Overview Of NERL-Cincinnati Products or Projects Related to Mining Issues

Publications and Presentations:

Hill, B.H., W.T. Willingham, L.P. Parrish, and B.H. McFarland. 2000. Periphyton community responses to elevated metal concentrations in a Rocky Mountain stream. Hydrobiologia (In press).

The effects of elevated metals on stream periphyton in the Eagle River, a mining impacted river in central Colorado, were assessed in 1991 and 1992 using assemblage information (taxa richness, community similarity) and non-taxonomic measures (biomass, chlorophyll *a*, autotrophic index). The number of periphyton genera collected ranged from 2 at a site adjacent to abandoned mining operations to 21 at a downstream site, but was not significantly correlated with dissolved metals concentrations. *Fragilaria* and *Achnanthes* were the dominant genera at all sites, with *Fragilaria* dominating the less impacted sites and *Achnanthes* dominating at the more impacted sites. Taxonomic similarity was greatest among those sites receiving the greatest inputs of metals from mining operations, where the coefficient of similarity ranged from 0.87 to 0.99. Cluster analyses revealed significant differences among sites adjacent to the mine and either the upstream or downstream sites. Chlorophyll *a* content of periphyton and the autotrophic index in both years showed significant downstream decreases associated with increasing dissolved metals concentrations. Overall, the periphyton community data were able to separate metal contaminated sites from reference or less impacted sites, and responded in predictable ways to increasing metal concentrations of Eagle River water.

Clements, William, H., Carlisle, D.M., Lazorchak, J.M. and Johnson, P.C., 2000. "The Role of Heavy Metals in Structuring Benthic Macroinvertebrate Communities in Colorado's Mountain Streams." Ecological Applications June 2000

Analysis of macroinvertebrate assemblages in relation to environmental gradients in Rocky Mountain streams. Michael B. Griffith, Philip R. Kaufmann, Alan T. Herlihy, and Brian H. Hill. Ecological Applications in press

Abstract. Using redundancy (RDA) and canonical correspondence analysis (CCA), we assessed relationships among chemical and physical characteristics and macroinvertebrate assemblages at stream sites sampled by the Regional Environmental Monitoring and Assessment Program (R-EMAP) in the mineral belt of the Southern Rockies Ecoregion in Colorado. We contrasted results of analyses where community structure was summarized as community metrics and analyses based on genera abundances. Our objective was to identify metrics or taxa diagnostic of major environmental stressors in these streams. When RDA was used to analyze the community metrics data, three axes were significant, accounting for 96% of the metric-environment relation. The first RDA axis was correlated with dissolved cadmium, sediment zinc, and total suspended solids, variables that indicate it was related to mining effects. The second and third RDA axes were correlated with water temperature, mean substrate embeddedness, mean canopy density at the banks, and a riparian human disturbance index for agriculture, variables associated with riparian and substrate alterations associated with grazing by livestock. When CCA was used to analyze the genera abundance data, four axes were significant, accounting for 45% of the species-environment relation. The CCA axes were correlated with total and dissolved iron, water temperature, dissolved and total organic carbon, mean bank height, and mean water surface gradient, variables associated with riparian disturbance from livestock grazing, but not with mining effects. Because CCA measures variation in community structure in terms of changes in the absolute abundances of different genera relative to one another, that analysis of genera abundances was sensitive to the effects of riparian disturbance and stream size, but not to the general toxicological effects associated with mining that reduced the abundances of all genera. Community metrics measure various aspects of community structure, including taxa richness, taxa relative abundances, and taxa relative dominance, and were sensitive to the effects of mining, riparian disturbance, and stream size. Some community metrics, such as the percent abundance of the most dominant taxon, the total number of individuals, the total number of taxa, and the number of chironomid taxa, may be used to diagnose the environmental stressors in these streams, while the results of the CCA for genera abundances may be used to design new metrics for this purpose.

"Metallothionein Gene Transcription as an Indicator of Metal Exposure in Fathead Minnows" Reddy, T.V., Lattier, D.L., Lazorchak, J. M., Smith, M.E., and Toth, G.P.

www.epa.gov/nerleerd/recentpres.html

Environmentally persistent metals,, are ubiquitous in surface waters, and have been shown to effect the viability and reproductive health of species living in these habitats. Metal induced transcription of specific genes is a sensitive exposure indicator, and directly correlates with an organism's biological state in real time. Expression of the metallothionein (*Mt*) genes, coding for a circulating cysteine rich, low molecular weight metal binding protein is regulated by numerous metals. Using available DNA sequences for common carp (*Cyprinus carpio*) metallothionein, we have carefully designed synthetic oligonucleotide primers which detect, in an RT-PCR scheme, *Mt* mRNA in both embryonic and adult Cyprinidae, fathead minnow (*Pimephales promelas)*. A modified 48-hr acute renewed method (see Table) was used to expose larval and adult fathead minnows to Zinc, Copper and Cadmium. Concentrations used for exposure were chosen from the literature using AQUIRE . Criteria used for selection of metal concentrations were less than 7-day old fish or adult fish 90 days or older, moderately hard water, and a concentration that was near the chronic No Observable Effect Level. Agarose gel electrophoresis of RT-PCR amplified products indicated cDNA corresponding to *Mt* message at the predicted molecular weight. Comparison of gene-specific products with the internal standards for 18S ribosomal RNA message, provided a means to quantify the relative levels of induced *Mt* mRNA. The expression of ribosomal RNA, which is the primary component of total RNA, is ostensibly invariant during cell cycle and between cell types; therefore, the relative level of rRNA remains constant from sample to sample. Ribosomal RNA has also been shown to be refractory to experimental treatment with numerous toxic and biochemical reagents. Use of the described monitoring scheme will enhance our knowledge of exposures, in teleost populations, to bioavailable metal compounds across a range of surface waters.

A.T. Herlihy, J.M. Lazorchak, F.V. McCormick, D.J. Klemm, M.E. Smith, W.T. Willingham, and L.P. Parrish Quantifying the Regional Effects of Mine Drainage on Stream Ecological Condition in the Colorado Rockies from Probability Survey Data - Results of the EPA Region 8 REMAP Project. Presented on 4/98 EMAP Symposium

The Southern Rockies Ecoregion contains almost 95% of the mineralized portion of the Rocky mountains. For the past century, strategic mining of metals has occurred in this area. Runoff from both active and inactive mining sites have contaminated waters and sediments. In 1993 and 1994, the U.S. EPA conducted a probability survey as part of its Regional Environmental Monitoring and Assessment Program (REMAP) in the Colorado portion of the Southern Rockies. The survey targeted second-fourth order streams as represented on digitized 1:100,000 scale maps. Over the two summers of the study, samples were successfully collected from 73 probability sites and 13 hand-picked reference (good) and test (bad) sites for indicators of fish and macroinvertebrate assemblages, physical habitat, and sediment and water chemistry and toxicity. The sample design allows inference to the status of 6,630 km of stream length in the Colorado Rockies from the sample data. Using stream chemistry, sites were classified into Least Disturbed, Mixed Impacts, and Mine Drainage Impacted Chemical Classes. The study area had roughly equal stream lengths in each of the three classes. Overall, an estimated 1,844 km (28%) of stream length had a sulfate signature of mine drainage, 438 km (7%) exceeded state Zn criteria and 376 km (6%) had water toxic to the test organisms. Sites with elevated metals and toxicity were concentrated in the mine drainage chemical class. Water column toxicity tests (48 hour fathead minnow and *Ceriodaphnia* survival) were better indicators of mine drainage stress than sediment toxicity test (7 day *Hyalella azteca*).

McFarland, B.H. and B.H. Hill. 1997. Abnormal *Fragilaria* spp. found in streams impacted by mine drainage. Journal of Freshwater Ecology 12: 141-149.

Periphytic diatom samples from a metal-contaminated Rocky Mountain river in Colorado, U.S.A. were analyzed on two occasions for the presence of morphological abnormalites. Samples were collected from natural (rocks) and artificial (tiles) substrates at 12 sites displaying a range of metal concentrations. Members of the genus *Fragilaria* sensu Krammer and Lange-Bertalot (including *Synedra* and *Hannaea*), which was abundant at all of the sampling sites, exhibited the highest incidence of abnormalities. There were no significant differences in percentage of deformed cells between natural and artificial substrates. Percentage of diatom abnormalities at each site ranged from $0.2 \pm 0.2\%$ to $12.0 \pm 2.0\%$ of the total population, and normal *Fragilaria* valves were always observed along with abnormal valves for each taxa. Percentage of abnormal valves at a site was transformed (arc sine square root) and regressed against dissolved cadmium, copper, iron, and zinc. For 1991, the best regression model fit (based on Mallows' C_p) was a two variable model using Cd and Zn (r^2=0.39, C_p=2.4). In 1992, the four variables model (Cd, Cu, Fe, and Zn) provided the best fit to the *Fragilaria* data (r^2=0.60, C_p=5.0). These data indicate that morphologically abnormal *Fragilaria* valves may be an indicator of elevated dissolved metal concentrations in streams.

Hill, B.H., J.M. Lazorchak, F.H. McCormick, and W.T. Willingham. 1997. The effects of elevated metals on benthic community metabolism in a Rocky Mountain stream. Environmental Pollution 96: 183-190.

The effects of elevated metals (dissolved ZN, MN and/orFe) in a Rocky Mountain stream were assessed using measures of primary productivity, community respiration, and water-column toxicity. Primary productivity was measured as rates of O_2 evolution from natural substrates incubated in closed chambers. Oxygen depletion within these chambers, when incubated in the dark, provided estimates or periphyton community respiration. Sediment community respiration on fine-grained sediments, collected and composited along each stream study reach, was measured on-site by incubating these sediments in closed chambers and measuring O_2 depletion. Toxicity was measured as percent mortality of *Ceriodaphnia dubia* during 48h acute tests. Gross (GPP) and net primary productivity (NPP) decreased significantly with increasing metal concentrations, from 10.88 ± 1.46 g O_2 m^{-2} d^{-1} to 0.83 ± 0.20 g O_2 m^{-2} d^{-1} and 9.85 ± 1.43 g O_2 m^{-2} d^{-1} to 0.81 ± 0.20 g O_2 m^{-2} d^{-1}, respectively for the reference and most impacted site. Community respiration (CR) declined from 0.65 ± 0.08 g O_2 m^{-2} d^{-1} to 0.02 ± 0.01 g O_2 m^{-2} d^{-1} with increasing metal concentrations. Sediment community respiration (SCR) decreased from 0.26 ± 0.02 g O_2 m^{-2} d^{-1} to 0.01 ± 0.01 g O_2 m^{-2} d^{-1} at these same sites. *Ceriodaphnia dubia* mortality increased from 0% at the reference site to 95 ± 5% at the most impacted sites. Net daily metabolism, quantum yield, and assimilation ratio all decreased with increasing metal concentrations suggesting that both autotrophic and heterotrophic components of the periphyton community were impaired. Overall, functional measures were able to discern sites receiving greater metal impacts from less-impacted sites, with combinations of dissolved metals explaining between 25 and 92% of the variance in the regression models. Using these regression models we were able to calculate lethal and inhibition concentrations of dissolved Zn in the Eagle River. The lethal concentration (LC_{50}) of Zn for *Ceriodaphnia dubia* is 123 μg/L. The concentrations of Zn which inhibited respiration (IC_{50}) were 177 μg/L for CR and 199 μg/L for SCR. These results indicate functional measures may be as sensitive to metal concentrations as acute toxicity tests.

W. Clements and D. Carlisle, Dept. of Fishery and Wildlife Biology, Colorado State Univ., Fort Collins, CO; J. Lazorchak, U.S. EPA, Cincinnatti, OH; P. Johnson, 12621 Alpine Dr., Anchorage, AK. Status and condition of Rocky Mountain streams: development of a regional biotic index to assess effects of heavy metals. Presented on 11/97 at Society of Environmental Toxicology and Chemistry. To assess status and condition of headwater streams in the Southern Rocky Mountain ecoregion, we selected 62 Colorado streams using a random probability sampling technique. Physicochemical characteristics, heavy metal concentrations, and benthic macroinvertebrate community structure were measured in all streams. Results of stepwise multiple regression indicated that 13 community metrics were significantly correlated with heavy metal concentration. These 13 metrics were assigned scores (1, 3, or 5) based on univariate analyses and a biotic index was calculated by summing the scores at each station. Results of one-way ANOVA showed that the index was sensitive to heavy metals and was significantly reduced in streams where metal levels exceeded 2x the U.S. EPA criterion value. We validated the index using independent data collected from the Arkansas River, a Colorado stream impacted by historic mining operations. The index was sensitive to seasonal changes in metal concentration in the Arkansas River and responded to improvements in water quality following remediation. Because the 62 streams in our study were randomly selected, our results have broad applicability for all headwater streams in the southern Rocky Mountain Ecoregion. To assess the status of streams in this region, the 62 streams were placed into one of four categories, based on metal concentrations and the biotic index. Results indicated that 63% of these streams were undisturbed or minimally disturbed, 23% were metal-polluted, 6% were buffered from metal pollution, and 8% were impacted by disturbances other than metals.

McCormick, F.H., B.H. Hill, L.P. Parrish, and W.T. Willingham. 1994. Mining impacts on fish assemblages in the Eagle and Arkansas Rivers, Colorado. Journal of Freshwater Ecology 9: 175-179.

Fish were collected at 18 sites in the Arkansas (N=6) and Eagle (N=12) rivers. Richness at all sites was low (1-3) species. Analyses of fish assemblage data from the Arkansas and Eagle rivers and their tributaries suggested significant differences among sites subject to mine impacts and control or recovering sites. Native taxa were collected at only one site in the Arkansas River drainage (*Onchrhynchus clarki clarki*) and only at the Eagle River control sites (Piaute sculpins, *Cottus beldingi*).

An Approach for Setting Restoration Goals in Mine Waste Impacted Watersheds Using Toxicity/Chemisty Profiles. Lazorchak, J.M.[2], Smith, M.E.[1], Willingham, W.T.[3], and Parrish, L[3]. [1]SBI Environmental, c/o U.S. EPA, 26 West Martin Luther King Dr., Cincinnati, 45268. [2] U.S. EPA, 26 West Martin Luther King Dr., Cincinnati, Ohio 45268. [3]U.S. EPA, Region VIII, Denver, Colorado, 80202. Presented at the 1997 Annual SETAC Meeting.

ABSTRACT

In the fall of 1995 and spring of 1997 the U.S. Environmental Protection Agency (U.S. EPA) Region VIII collected physicochemical and toxicity information from the Clear Creek watershed in central Colorado. The purpose of this investigation was to evaluate the relative advantages of an ecotoxicological approach for identifying residual contaminant sources and evaluating established clean up goals within a watershed. *Ceriodaphnia* and fathead minnow 48-hr acute toxicity tests and metal analyses were performed on 32 stream samples collected in 1995 and 37 stream samples collected in 1997 from the Clear Creek watershed. Stream water was shipped overnight to the NERL Cincinnati Laboratory for toxicity testing and to the U.S. EPA Laboratory for metal analyses. Both profile tests(100% stream water) and definitive toxicity tests (stream samples serially diluted with moderately hard reconstituted water) were performed. *Ceriodaphnia* toxicity results (LC50s and 100% stream water) in 1995 and 1997 showed a similar trend throughout the watershed; the upper 20 miles of the mainstem of Clear Creek had LC50s >20% stream water, while the lower 20 miles had LC50s <10% stream water. Converting the toxicity results to a no observed acute effect level (NOAEL), in metal concentrations, will be presented as an alternative approach for evaluating clean up goals within an entire watershed.

INTRODUCTION

Most aquatic ecotoxicologists believe that in order to manage aquatic ecosystems one must, with some level of confidence, know the biological status of the system to be managed. This is especially true if one of the management objectives is to maintain and/or restore the biological integrity of that system. One aspect of aquatic ecosystem management concerns the evaluation of chemical stressors in the system. The importance of biological endpoints in this aspect of management is captured by Cairns and Mount (1990): " No instrument has yet been devised that can measure toxicity! Chemical concentrations can be measured with an instrument but only living material can be used to measure toxicity." Aquatic ecologists and toxicologists believe that in order to adequately manage aquatic ecosystems, both the chemical, and biological status of the system needs to be measured. Ecotoxicologists understand that organisms respond to the totality of their environment and that simply defining that environment by measuring selected physical and chemical parameters is inadequate for two reasons. First, with chemical measures you only find what you are analyzing for, and second, even if all the chemical constituents could be measured, our understanding of the interactions of toxicants (additivity, synergism, antagonism, etc.) is simply inadequate to predict biological responses. To address such issues the U.S. Environmental Protection Agency (U.S. EPA) started the Whole Effluent Toxicity Testing (WETT) program (U.S. EPA, 1991). WETT requires all major NPDES discharges to monitor their effluents using at least three species and/or have permit limitations. Traditional acute and chronic toxicity tests were recommended. In October 16, 1996, the U.S. EPA required that only certain standardized agency methods be used for setting monitoring requirements. A similar approach is proposed for ecorestoration of streams. However, actual stream water will be used for monitoring and setting restoration goals.

In the fall of 1995 and spring of 1997 the U.S. Environmental Protection Agency (U.S. EPA) Region VIII collected physicochemical and toxicity information from the Clear Creek watershed in central Colorado. The purpose of this investigation was to evaluate the relative advantages of an ecotoxicological approach for identifying residual contaminant sources and evaluating established clean up goals within a watershed. *Ceriodaphnia* and fathead minnow 48-hr acute toxicity tests and metal analyses were performed on 32 stream samples collected in 1995 and 37 stream samples collected in 1997 from the Clear Creek watershed. Stream water was shipped overnight to the NERL Cincinnati Laboratory for toxicity testing and to the U.S. EPA Laboratory for metal analyses. Both profile tests (100% stream water) and definitive toxicity tests (stream samples serially diluted with moderately hard reconstituted water) were performed. *Ceriodaphnia* toxicity results (LC50s and 100% stream water) in 1995 and 1997 showed a similar trend throughout the watershed; the upper 20 miles of the mainstem of Clear Creek had LC50s >20% stream water, while the lower 20 miles had LC50s <10% stream water. Converting the toxicity results to a no observed acute effect level (NOAEL), in metal concentrations, will be presented as an alternative approach for evaluating clean up goals within an entire watershed.

Materials and Methods

Sample Collection and Dissolved Metals Analysis

Samples were collected in the field using a 10L polyethylene bucket, rinsed with site water prior to sample collection. After collection, the samples were split into separate aliquots for toxicity and chemical analysis.

The toxicity samples were collected in labeled 4L cubitainers, rinsed with sample water. These samples were stored on ice in the field, as well as during sample shipment. The samples were shipped overnight, with sufficient ice to insure unmelted ice existed on arrival.

Dissolved metals samples were collected by vacuum filtering the samples through a 0.45 micron disposable filter, one filter used for each site. The samples were stored in 1L cubitainers and preserved using 2 ml concentrated nitric acid. Metals analysis was performed based on SOP's developed in-house for use by USEPA Region VIII and the Regional contractor.

Sample Receipt and Toxicity Testing

On arrival, the pH, alkalinity, hardness, dissolved oxygen, conductivity, and temperature were measured for each toxicity sample. Then an aliquot of sample was collected for initiating the toxicity tests and the rest were stored at 4°C for test renewal or restart.

Toxicity tests were 48 hour renewed, acute tests using less than 24 hour old *Ceriodaphnia dubia* and 3 to 5 day old *Pimephales promelas*. The tests were conducted using in-house SOP's developed for use by NERL. Tests were conducted at 20°C.
All tests were initiated within 36 hours of sample collection. If the dilution series for a test was inappropriate, i.e. excess mortality in a low concentration or insufficient mortality in a high concentration (excluding 100%), the test was restarted.

LC50 values were determined using Trimmed Spearman-Karber. Survival NOAEL values were determined using an ANOVA and Dunnett's Multiple Comparison Method.

The NOAEL adjusted metals levels were determined by multiplying the dissolved metal level for a site by the *C. dubia* (or *P. promelas*) percent NOAEL value for that site. In some cases, the survival in the lowest test concentration was different from the control survival. When this occurred, an estimated NOAEL was established. If the survival in the lowest test concentration was greater than 50%, the NOAEL was estimated to be one lower dilution. If the survival was less than 50%, the NOAEL was estimated as two dilutions lower.

Clear Creek Mainstem Sites

River miles are measured from the confluence of Clear Creek with the South Platte River.

SW-01 Clear Creek at the USGS gage near Golden. River Mile = 18.4
SW-02A Clear Creek downstream from North Fork, approximately ½ mile downstream from the downstream side of Tunnel #2. River Mile = 24.6
SW-02 Clear Creek approximately ¼ mile downstream from the confluence with the North

Fork of Clear Creek at the intersection of highways 6 and 119. River Mile = 30.6

SW-03 Clear Creek upstream from the North Fork, approximately one mile upstream from Tunnel #6. River Mile = 33.24

SW-04A Clear Creek downstream of Idaho Springs WWTP near where the old Colorado DOW Game Check Station was located. River Mile = 36.44

SW-04B Idaho Springs WWTP discharge at confluence with Clear Creek.

SW-05 Clear Creek approximately 50 meters downstream from the ARGO tailings and Tunnel. River Mile = 38.14

SW-07A Clear Creek 100 meters upstream of the ARGO Tunnel discharge. Station added 3/97. River Mile = 38.58

SW-07 Clear Creek approximately 150 meters downstream from Chicago Creek. River Mile = 39.14

SW-10 Clear Creek upstream of confluence with Chicago Creek. River Mile = 39.38

SW-13 Clear Creek upstream from Idaho Springs at Stanley Road turnoff. Station is downstream of Trail Creek but upstream of the Big-5. River Mile = 39.98

SW-21 Clear Creek at the Lawson USGS gage. River Mile = 45.28

SW-23 Clear Creek upstream from the confluence with West Fork of Clear Creek. River Mile = 47.48

SW-24A Georgetown WWTP discharge at confluence with Clear Creek.

SW-26 Clear Creek ups of confluence with South Fork at bridge in Silver Plume. River Mile = 53.68

SW-28 Clear Creek upstream from Quayle Creek. River Mile = 58.03

Clear Creek Tributaries:

River miles are measured from the confluence of Clear Creek with the South Platte River.

SW-06 ARGO Tunnel discharge near confluence with Clear Creek. River Mile = 38.54

SW-08A Chicago Creek near confluence with Clear Creek. River Mile = 39.34

SW-12 Big-5 Tunnel near confluence with Clear Creek. River Mile = 39.88

SW-14 Trail Creek approximately 100 meters upstream from confluence with Clear Creek. River Mile = 41.08

SW-15 Fall River approximately ¼ mile upstream from confluence with Clear Creek. River Mile = 41.38

SW-17 Rockford Tunnel near mouth to Clear Creek. River Mile = 41.68

SW-27A Smaller 4-inch pipe just downstream of Burleigh Tunnel outfall. River Mile = 53.98

SW-27 Burleigh Tunnel outfall near Clear Creek (6-inch pipe). River Mile = 53.99

SW-59 Quayle Creek near confluence with Clear Creek in Bakerville. River Mile = 57.99

North Fork Clear Creek:

River miles are measured from the North Fork's confluence with Clear Creek.

SW-36 North Fork of Clear Creek near confluence with Clear Creek just upstream from gage station. River Mile = 0.16 (From confluence with South Platte, River Mile = 30.64)

SW-37 North Fork of Clear Creek downstream from Russell Gulch. River Mile = 3.2

SW-39 North Fork of Clear Creek approximately ¼ mile downstream from Blackhawk

WWTP discharge. River Mile = 5.94

SW-40 North Fork of Clear Creek upstream from the Blackhawk WWTP discharge and downstream from National Tunnel. River Mile = 6.54

SW-43 North Fork of Clear Creek approximately 100 meters downstream from Gregory Gulch. River Mile = 7.08

SW-48 North Fork of Clear Creek approximately 150 meters downstream from Chase Gulch. River Mile = 7.32

SW-49B North Fork of Clear Creek downstream from Silver Creek. River Mile = 9.46

North Fork Clear Creek Tributaries:

River miles are measured from the North Fork's confluence with Clear Creek.

SW-38 Russell Gulch near mouth. River Mile = 3.24

SW-41 National Tunnel near mouth. River Mile = 6.58

SW-42 Four Mile Gulch near mouth. River Mile =

SW-44 Gregory Gulch near mouth. River Mile = 7.12

SW-46 Gregory Incline near mouth. River Mile = 7.16

SW-47 Chase Gulch near mouth. River Mile = 7.36

West Fork Clear Creek:

River miles are measured from the West Fork's confluence with Clear Creek.

SW-29 West Fork of Clear Creek approximately 100 meters upstream from the confluence with Clear Creek. River Mile = 0.04 (From confluence with South Platte, River Mile = 47.18)

SW-29A Empire WWTP discharge at confluence with the West Fork of Clear Creek.

SW-30 West Fork of Clear Creek upstream from Empire WWTP discharge. River Mile = 0.98

SW-33A West Fork of Clear Creek approximately ½ mile downstream from SW-33 and Blue Creek. Station is located 3 miles west of Empire, 200 feet east of the forest boundary sign and ¼ mile down the road from the wire frame gate. River Mile = 3.58

SW-35 West Fork of Clear Creek upstream from Woods Creek. Station is easily accessed and sampled near road crossing. River Mile = 9.28

West Fork Clear Creek Tributary:

SW-34 Woods Creek upstream from confluence with West Fork of Clear Creek. River Mile = 9.08 from West Fork confluence with Clear Creek

South Fork Clear Creek:

SW-25A South Fork near confluence with Clear Creek. River Mile = 0.04 (From confluence with the South Platte, River Mile = 51.48)

SW-25 South Fork of Clear Creek upstream from the old ore milling site. River Mile = 0.44 (from confluence with Clear Creek)

RESULTS

Table 1. *Ceriodaphnia* Toxicity and Stream Chemistry Data from Mainstem Clear Creek, 10-95 & 4-97.

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97
SW-01	<12.5 17.7	6.25* 12.5	11 10	572 340	1007 720	79 93	40 44
SW-02A	<12.5 N/S	6.25* N/S	10 N/S	582 N/S	1054 N/S	77 N/S	39 N/S
SW-02	10.2 14.4	6.25 3.125*	18 12	658 328	1232 965	78 98	38 41
SW-03	6.9 15.9	3.125* 6.25	13 12	529 516	954 1040	64 89	37 42
SW-04A	7.6 10.0	3.125* 6.25	14 14	577 612	1030 1190	65 94	36 44
SW-05[A]	7.0 10.2	3.125* 6.25	14 15	538 539	858 871	63 86	36 41
SW-07	15.5 33.4	6.25 12.5*	13 10	214 234	198 172	54 74	41 43
SW-10	51.8 N/S	50 N/S	12 N/S	248 N/S	218 N/S	58 N/S	42 N/S
SW-13	43.8 58.9	25 25	14 10	234 298	206 174	57 84	41 46
SW-21	>100 70.7	50 50	1.1 5.0	134 112	148 190	58 81	45 47
SW-23	>100 64.4	50 50	1.0 1.6	213 337	48 19	60 85	48 52
SW-26	21.0 29.4	6.25* 12.5	0.7 1.1	682 989	32 38	57 88	47 50
SW-28[B]	>100 >100	100 100	0.5 1.7	<4 <20	10 19	39 79	37 42

A) SW-04B discharges below this station. B) SW-24A discharges below this station.
* indicates estimated (calculated) NOAEL values. Red Indicates Background Station

Table 1A. *Ceriodaphnia* Percent NOAEL Adjusted Chemistry Data from Mainstem Clear Creek, 10-95 & 4-97.

	Cu	Zn	Mn
SXS	µg/l	µg/l	µg/l
	10/95 4/97	10/95 4/97	10/95 4/97
SW-01	0.7 1.0	36 43	63 90
SW-02A	0.6 N/S	36 N/S	66 N/S
SW-02	1.1 0.4	41 10	77 30
SW-03	0.4 1.0	17 32	30 65
SW-04A	0.4 0.1	18 38	32 74
SW-05	0.4 1.0	17 34	27 54
SW-07	1.0 1.0	13 29	12 22
SW-10	6.0 N/S	124 N/S	109 N/S
SW-13	4.0 3.0	59 75	52 44
SW-21	0.5 3.0	67 56	74 95
SW-23	0.5 0.8	107 170	24 10
SW-26	D.L. 0.1	43 124	2 5
SW-28	0.5 1.7	D.L. D.L.	10 19

Red Indicates Background Station
Average Adjusted Metal Values are derived as follows:
% *Ceriodaphnia* NOAEL X Dissolved Metal Value

Table 2. *Ceriodaphnia* Toxicity and Stream Chemistry Data from Clear Creek Tributaries and Contaminant Sources, 10-95 & 4-97.

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97
SW-06	<1.56 0.13	0.039 0.039*	7739 4100	52960 46800	98090 98100	1294 1274	N/A N/A
SW-08A	>100 >100	100 100	1.0 1.6	85 96	83 92	30 37	28 28
SW-12	N/S 0.16	N/S 0.078	N/S 1530	N/S 9120	N/S 27500	N/S 1407	N/S N/A
SW-14	0.5 4.2	0.187* 3.0	176 87	1122 944	493 237	104 101	4.0 N/A
SW-15	>100 >100	100 100	5.0 3.7	24 28	19 10	29 38	23 25
SW-17I	N/S 0.96	N/S 0.75	N/S 904	N/S 2850	N/S 8040	N/S 421	N/S N/A
SW-17O	N/S 0.46	N/S .375	N/S 1420	N/S 3400	N/S 9890	N/S 472	N/S N/A
SW-27A	N/S 0.22	N/S 0.094	N/S <1.0	N/S 31300	N/S 4050	N/S 423	N/S 170
SW-27	N/S 0.24	N/S 0.094	N/S 6.9	N/S 65400	N/S 2910	N/S 407	N/S 108
SW-59	>100 70.1	50 50	0.5 1.1	74 68	<2 <3	52 56	42 44

* indicates estimated (calculated) NOAEL values.

Table 2A. *Ceriodaphnia* Percent NOAEL Adjusted Chemistry Data from Clear Creek Tributaries and Contaminant Sources, 10-95 & 4-97.

	Cu	Zn	Mn
SXS	µg/l	µg/l	µg/l
	10/95 4/97	10/95 4/97	10/95 4/97
SW-06	3 2	21 18	38 38
SW-08A	1 2	85 96	83 92
SW-12	N/S 1	N/S 4	N/S 11
SW-14	0.3 3	2 28	1.0 0.3
SW-15	5 4	24 28	19 10
SW-17I	N/S 7	N/S 21	N/S 60
SW-17O	N/S 5	N/S 13	N/S 37
SW-27A	N/S D.L.	N/S 29	N/S 4
SW-27	N/S D.L.	N/S 61	N/S 3
SW-59	0.3. D.L.	37 34	D.L. D.L.

Average Adjusted Metal Values are derived as follows:
% *Ceriodaphnia* NOAEL X Dissolved Metal Value

Table 3. *Ceriodaphnia* Toxicity and Stream Chemistry Data from North Fork Clear Creek, 10-95 & 4-97.

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97
SW-36	14.2 4.5	6.25 3.125	15 10	1221 917	2735 813	187 143	19 15
SW-37	12.4 N/S	3.125 N/S	22 N/S	2039 N/S	4010 N/S	185 N/S	7 N/S
SW-39	3.8 7.6	1.56 6.25	52 20	3875 1070	8343 2690	270 154	8 11
SW-40	17.1 24.9	12.5 25.0	67 11	1902 467	4284 1260	178 96	7 17
SW-43	5.9 3.1	3.125 1.56	207 23	1352 458	2985 1090	132 77	10 12
SW-48	40.7 70.7	25 25	10 5	382 180	245 121	42 40	28 25
SW-49B	>100 >100	100 100	1 2	<4 <20	25 6	22 27	22 22

Red Indicates Background Station

Table 3A. *Ceriodaphnia* Percent NOAEL Adjusted Chemistry Data from North Fork Clear Creek, 10-95 & 4-97.

	Cu	Zn	Mn
SXS	µg/l	µg/l	µg/l
	10/95 4/97	10/95 4/97	10/95 4/97
SW-36	1 0.3	76 29	171 25
SW-37	1 N/S	64 N/S	125 N/S
SW-39	1 1	60 67	130 168
SW-40	8 3	238 117	534 315
SW-43	6 0.4	42 7	93 17
SW-48	3 1	96 45	61 30
SW-49B	1 2	D.L. D.L.	25 6

Red Indicates Background Station

Average Adjusted Metal Values are derived as follows:
% *Ceriodaphnia* NOAEL X Dissolved Metal Value

Table 4. *Ceriodaphnia* Toxicity and Stream Chemistry Data from North Fork Clear Creek Tributaries and Contaminant Sources , 4-97.

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
SW-38	7.4	6.25	30	952	522	193	13
SW-41	0.75	0.375	31	6420	18800	717	N/A
SW-44	0.9	0.75	51	1490	937	144	12
SW-46	<1.56	0.78*	755	6580	28400	359	N/A
SW-47	13.8	3.125*	15	1530	154	96	50

* indicates estimated (calculated) NOAEL values.

Table 4A. *Ceriodaphnia* Percent NOAEL Chemistry Data from North Fork Clear Creek Tributaries and Contaminant Sources, 4-97.

	Cu	Zn	Mn
SXS	µg/l	µg/l	µg/l
SW-38	2	60	33
SW-41	0.1	24	71
SW-44	0.4	11	7
SW-46	6	51	222
SW-47	0.5	48	5

Average Adjusted Metal Values are derived as follows:

% *Ceriodaphnia* NOAEL X Dissolved Metal Value

Table 5. *Ceriodaphnia* Toxicity and Stream Chemistry Data from West Fork Clear Creek, 10-95 & 4-97.

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97
SW-29	>100 >100	100 100	5 10	22 30	349 392	52 74	38 44
SW-30[C]	>100 N/S	100 N/S	4 N/S	20 N/S	385 N/S	52 N/S	37 N/S
SW-33A	>100 N/S	100 N/S	1 N/S	15 N/S	340 N/S	56 N/S	45 N/S
SW-35	>100 >100	100 100	1 2	35 36	192 183	35 55	23 28

C) SW-29A discharges below this station. Red Indicates Background Station

Table 6. *Ceriodaphnia* Toxicity and Stream Chemistry Data from West Fork Clear Creek Tributary, 10-95 & 4-97.

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97
SW-34	>100 >100	100 100	1 6	24 78	1405 1910	92 148	81 87

<u>Table 7. *Ceriodaphnia* Toxicity and Stream Chemistry Data from South Fork Clear Creek, 10-95 & 4-97.</u>

	LC50	NOAEL	Cu	Zn	Mn	HDNS	ALK
SXS	(%)	(%)	µg/l	µg/l	µg/l	mg/l	mg/l
	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97	10/95 4/97
SW-25A	>100 >100	100 100	1 2	57 58	24 6	48 59	39 47
SW-25	>100 >100	100 50*	0.7 <1	34 39	11 6	59 60	49 50
SW-25^	>100	50*	D.L.	20	3	60	50

^ % NOAEL Adjusted Chemistry Data for SW-25, 4-97.

<u>NOTE</u>

N/S indicates sites not sampled a particular year.

N/A indicates chemical analysis values that could not be determined

D.L. indicates chemical analysis values below the detection limit of the method.

* indicates estimated (calculated) NOAEL values.

CONCLUSIONS:

Using a Site Specific Toxicity (SST) approach to derive instream ecorestoration goals appears promising.

The SST approach may be more relevant than using National Water Quality Criteria since site water characteristics are taken into account during testing.

Ceriodaphnia NOAEL levels from site water tests are within a factor of two of the National Water Quality Criteria Levels calculated for the Clear Creek Mainstem watershed.

A *Ceriodaphnia* Watershed Ecorestoration goal of 50 ug/L of Zinc is below the National Chronic Criteria of Zinc, 82 ug/L. However, using a $\pm$ 1 SD approach results in a range around the National Criteria, 5 - 95 ug/L.

The Fathead Minnow Watershed Ecorestoration goal of 332 ug/L of Zinc is higher than the

Acute National Criteria for Zinc, 90 ug/L. $\pm$ 1 SD approach, 142 - 522 ug/L results in a range above the acute National Criteria.

The final approach chosen will depend on how the ecosystem responds to restoration. Site Specific Toxicity Restoration goals need to be calibrated by seeing how the instream biota respond. A Site Specific Biocriteria approach should be used to calibrate SST goals.

Reports

Toxicity Test Report
Task 4a. Conduct toxicity tests on Mine Waste Samples from 3-5 Technologies

Date: August 25, 1999 (Revised 4/11/00)
Project: Butte, Montana Mine Sites Toxicity Tests

Introduction

Water samples from four mine sites in the Butte, MT vicinity were shipped to the U.S. EPA Laboratory in Cincinnati, Ohio. A series of acute aquatic toxicity tests with *Pimephales promelas*, the fathead minnow, and *Ceriodaphnia dubia*, a freshwater invertebrate, and chronic aquatic toxicity tests with *Daphnia magna*, a freshwater invertebrate, were conducted on these samples. The purpose of these tests was to establish the level of toxicity for the discharge from the different mines sites and to evaluate the effectiveness of the treatment processes currently being used at these sites.

Definitions

Acute toxicity test: A test method that uses a short exposure period (i.e. 48 hours) to determine the lethal effects of an effluent or receiving water to a selected test organism.

Chronic toxicity test: A test method that uses an exposure period that is less than a complete life cycle (i.e. 4-7-days) to determine both the lethal and sub-lethal effects of an effluent or receiving water to a selected test organism. The sub-lethal effects can include growth or reproduction.

Definitive test: A test that uses a series of effluent or receiving water dilutions to determine the level of acute or short-term chronic toxicity a sample exhibits to a selected test animal.

Profile sample: A sample that is tested using only the 100% (undiluted) test sample.

No Observed Acute Effect Level (NOAEL): That concentration or percent sample in an acute test where the survival of the test animals is determined to not be statistically different from the survival of the control animals. If survival in the lowest test concentration is determined to be statistically different from the control, the data are evaluated to see if the survival in the lowest test concentration is greater than 40%. If it is, the assumption is made that the next dilution in the series would have survival not different from that of the control and this estimated data point is used as the NOAEL.

No Observed Effect Concentration: That concentration or percent sample in a short-term chronic test where the survival, growth or reproduction of the test animals is determined to not be statistically different from the survival, growth or reproduction of the control animals.

Fifty Percent Lethal Concentration (LC50): The estimated concentration of a compound or percent effluent or receiving water that would cause 50% mortality to the test animals.

Inhibition Concentration 25 (IC25): The estimated concentration of a compound or percent effluent or receiving water that would cause a 25% effect (reduction) in the reproduction or growth of the test animals.

Percent minimum significant difference (MSD%): The lowest percent difference between a control and effluent sample that an analysis of variance test (i.e. Dunnett's) can detect as being statistically different.

Methods

Samples were collected on Monday 7-7-99 and Tuesday 7-8-99 using a plastic graduated cup and 1 gallon cubitainers. Approximately 2 quarts were collected from each effluent, waste pile, reactor or receiving stream. After each sample was collected, air was depressed from each cubitianer, it was capped and placed in a cooler with 10 lbs of ice and shipped overnight to the EPA facility in Cincinnati. All coolers were received in good condition with all seals intact, and all samples were in acceptable condition. A total of six water samples collected from Calliope Mine were received on 7-7-99 and eight water samples collected from Crystal Mine, Lilly/Orphan Boy Mine and Peerless Mine were received on 7-8-99.

The most recent monthly chemical analyses were obtained from MSE and used to estimate what concentrations would be tested for each sample. Table 1 summarizes the chemistry of a selected number of metals used to determine the highest dilution to be tested and whether a Definitive test (dilution test) or Profile test (no dilution) would be performed. A zinc concentration of 150 µg/l was used as a guide to establish the highest dilution to run for both the definitive and profile tests. The acute toxicity of zinc is around 150 µg/l as long as the sample contains no complexing organics or high levels of carbonate.

Routine initial chemical parameters (Table 2) were determined and toxicity tests were started on arrival of the samples. The tests with *P. promelas* and *C. dubia* were 48-hr, renewed, acute tests, conducted at 20°C. Each sample was analyzed using both species, with the exception of Calliope Columns Out, which was analyzed using just the *C. dubia* acute test. In addition, nine samples were analyzed using a *D. magna* 4-day, growth and survival test, to provide a measure of the sensitivity of this method versus the two acute methods, as well as to provide a subsample of chronic test data for these sites. These included, Calliope Columns In, Influent Below Grade, Columns Out, Outflow Below Grade with treatment, Outflow Below Grade without Treatment, and Outflow Above Grade with pretreatment, Crystal Mine, Peerless Flow from Pile and Lilly Orphan Boy PT 3a.

All tests were conducted using moderately hard reconstituted water as the control and dilution water. Test conditions for *C. dubia, P. promelas*, and *D. magna* are contained in Tables 3, 4 and 5, respectively.

All LC50 values were determined using the 1994 U.S. EPA Toxicity Data Analysis Software that contains the Trimmed Spearman-Karber, version 1.5, which adjusts for control mortality. This software also was used to calculate the acute survival No Observed Acute Effect Level (NOAEL), the chronic survival No Observed Effect Concentration (NOEC) and the chronic growth NOEC using Dunnett's, version 1.5, and the IC25 values using ICp version 2.0.

Results and Discussion

All tests were successfully completed with acceptable control survival (90% or greater) or growth (250 mg for fatheads and 10X the initial weight for *D. magna*) for all tests. *C. dubia* restarts were required for Calliope IBG and Lilly Orphan Boy Pt 3b, due to high mortality in the low test concentrations. In addition, the Peerless FIP *C. dubia* test should have been restarted, due to 100% mortality in the lowest test concentration, but insufficient test sample was left to rerun another dilution series. Fish restarts were required for Calliope IBG, Peerless FFP and Lilly Orphan Boy Pt 3b. The mortality of the fish in the high concentrations for these samples was not sufficient to determine an LC50 or an NOAEL. No *D. magna* tests were restarted. The results for the *C. dubia* tests are contained in Table 6, the fish results are contained in Table 7 and the *D. magna* results are contained in Table 8. Tables 9, 10, 11 and 12 contain summaries of all routine initial and final chemistries. Table 13 presents the chemical analyses of a select number of metals that were analyzed by ICP. A full lab report of the ICP analyses is also attached. **Note the concentrations measured represent the concentration at the time of the test not at time of collection.** A subsample was collected at the time the tests were run and acidified with Nitrex. The samples were then filtered before they were analyzed by ICP.

Calliope Results

Results from Calliope indicate both the Columns In (CI) sample and the Influent Below Grade (IBG) sample were toxic to all three species. For CI, the *C. dubia* had an LC50 value of 0.07%, with an NOAEL of 0.05%, the *P. promelas* LC50 was 0.99%, with an NOAEL of 0.625% and the *D. magna* had an IC25 value of 0.349%, with a growth NOEC <0.3125%. For IBG, the *C. dubia* LC50 was 0.23%, with an NOAEL of 0.05%, the *P. promelas* LC50 was 9.2%, with an NOAEL of 1.25% and the *D. magna* IC25 was 1.58%, with a growth NOEC of 2.5%. The results from the four profile (100% concentration only) samples (CO, R2, R3 and R4) for Calliope indicate they did not cause significant toxicity, or a reduction in growth, to the test animals. In fact, the growth of the *D. magna* in the four profile samples exceeded the growth of the *D. magna* in the control sample (Table 7). In summary, both CI and IBG were found to exhibit acute toxicity to *C. dubia* and *P. promelas* and chronic toxicity to *D. magna*. It was determined the four profile samples did not cause significant mortality to any species and in fact exposure to the profile samples enhanced the growth of the *D. magna*.

Using the NOAEL for *C. dubia* of 0.05% for the IBG sample, a predicted safe Zn concentration of 3.2 μg/l can be obtained, i.e. 0.0005 x 6,430 (Table 13) = 3.2. For the same sample, the NOAEL for *P. promelas* is 1.25%, which results in a safe predicted zinc level of 80.4 μg/l. An actual measured chronic level can be generated from the *D. magna* growth results, using the NOEC of 1.58% we find a safe Zinc level of 101.6 μg/l. So an acceptable clean up level should be between 3 and 101.6 µg/l. Looking at the effluent from each of the reactors then we find the following:

R2 acceptable zinc concentration is 590 µg/l (Table 13) for all species tested, which is a 90.8% reduction in zinc concentration from the influent concentration and 184 times higher than the predicted safe level (590 ÷ 3.2 = 184) NOAEL for *C. dubia*.

R3 acceptable zinc concentration is 1,230 µg/l for all species tested which is 80.9% reduction from the influent concentration and 384 times higher than the NOAEL calculated for *C. dubia*.

R4 acceptable zinc concentration is 1,370 µg/l for all species tested which is a 78.7% reduction from the influent concentration and 436 times higher than the NOAEL calculated for *C. dubia*.

An observation that can be drawn from these results is that there is a high amount of organic material that is binding or complexing the metals and not allowing them to be bioavailable.

For the column study the NOAEL for *C. dubia* in the Column influent (CI) would predict a safe zinc concentration of 133 µg/l (0.05% x 26,600 µg/l). There is virtually no zinc in the effluent from the column (CO) so the column study looks very promising. One recommendation is to run the column faster and see how much zinc comes through and rerun the toxicity tests as an evaluation tool.

Crystal Mine Results

The results from Crystal Mine show that *C. dubia* had an LC50 of 0.04%, with an NOAEL of 0.016%, the *P. promelas* had an LC50 of 0.37%, NOAEL of 0.25% and the *D. magna* had an IC25 of 0.25%, with a growth NOEC of 0.0313%. It should be noted the *C. dubia* NOAEL was estimated. Survival in the lowest test concentration, 0.0313%, was 70%, which was determined to be different from the control for survival. The spread of the data indicates that survival in a 0.016% sample would be 80% or greater, so this value is supplied as the NOAEL, but it should be used with caution.

Using the estimated NOAEL for *C. dubia* we predict a safe level for zinc from this effluent would be 8.5 µg/l (0.016 x 53,000 µg/l = 8.48). *D. magna* NOEC predicts an acceptable zinc concentration of 16.6 µg/l, which is about twice as high as the NOAEL for *C. dubia*. Since this zinc level is so low other metals like Cd might be adding to the toxicity. Cd was highest in the Crystal mine sample over any other mine by a factor of 10.

Peerless Mine Results

Results for Peerless Portal (PP) show the *C. dubia* LC50 was 1.2%, with an NOAEL of 0.78% and the *P. promleas* LC50 was 39.2% with an NOAEL of 12.5%. For Peerless Flow into

Pile (FIP), the *C. dubia* LC50 and NOAEL were both less than 6.25%, due to 100% mortality in the lowest test concentration (6.25%). This test should have been restarted, but there was insufficient sample for a second test. The FIP results for *P. promelas* show an LC50 of 8.1% and an NOAEL of <6.25%, again below the lowest test concentration. All indications are this sample should have been tested using the concentration series used for PP. The results from Peerless Flow from Pile (FFP) show a *C. dubia* LC50 of 1.6%, with an NOAEL of 0.625%. The *P. promelas* LC50 was 28.3%, with an NOAEL of 10% and the *D. magna* IC25 was 2.24%, with a growth NOEC of 1.25%. All samples were acutely toxic, with the *C. dubia* being more sensitive than the fish. Sample FFP also exhibited chronic toxicity to the *D. magna*, with survival and growth in one or more of the test concentrations being significantly different from the control.

Using the NOAEL for *C. dubia*, a safe zinc level for the Peerless Portal (PP) would be 13.1 µg/l (0.078 x 1680). Using the NOAEL for *C. dubia* for the flow from the pile to estimate a safe level for the flow into the pile as well, 47.3 µg/l was calculated for the flow from the pile and
31.8 µg/l was calculated for the flow into the pile.

Lilly Orphan Boy Results

The upstream profile sample for Lilly Orphan Boy had *C. dubia* and *P. promelas* LC50 and NOAEL values >100%. The downstream sample had a *C. dubia* LC50 of 33.9%, with an NOAEL of 25% and the *P. promelas* had an LC50 of 70.7% with and NOAEL of 50%. Two samples were collected at the Pt 3 discharge, Pt 3a and Pt 3b. For Pt 3a, the *C. dubia* LC50 was 0.34%, with an NOAEL of 0.25%, the *P. promleas* LC50 was 3.0%, with an NOAEL 1.0% and the *D. magna* IC25 was 0.653%, with a growth NOEC of 1.0%. For Pt 3b, the *C. dubia* LC50 was 0.24%, with an NOAEL of 0.125% and the *P. promelas* LC50 was 6.1%, with an NOAEL of 4.0%.

The upstream sample shows no toxicity to either species used, while the downstream sample was toxic to both species. Using the Downstream NOAEL for *C. dubia* a safe level for zinc would be 70 µg/l (0.25 x 280). For the portal samples, Pt3a and Pt3b, the C. dubia NOAEL's were 0.25% and 0.125% respectively. This would result in safe zinc levels of 38 µg/l and 18 µg/l. The low zinc values would tend to indicate that the alkalinity and hardness are low in both samples. Looking at Table 2, the results from the routine chemical analysis of the initial samples for Pt3a and Pt3b show hardness and alkalinity could not be measured and the downstream sample only has a hardness and alkalinity of 20 mg/l. Thus, very little complexing is available.

TABLE 1. Recent Chemical Analyses at Mines in the Butte Area Used to Determine Test Concentrations

Mine/location	Al (Metal Conc µg/l)	Cu	Zn	Mn	Pb	Type Of Test	Test Dilution % sample	Sample ID
Calliope 05/05/99								
Influent Below Grade w/	14,100	3,050	11,100	3,700		Definit	5%	C2
Outflow Below Grade w/o	48	43	459	2,100		Definit	100%	C1
Above Grade pretreat	29	37	194	454		Definit	100%	C4
Below Grade with treat	14	8	249	661		Definit	100%	C3
Columns In								CI
Columns Out								CO
Peerless 04/02/99								
Portal	13	85	157	3,870	34	Definit	100%	PP
flow into Pile	12	8	551	36	34	Definit	100%	P1
flow from Pile	2,080	873	6,360	14,600	45	Definit	10%	P2
Lilly Orphan Boy 05/19/99								
PT3	3,100	142	15,700	6,050		Definit	4%	L1 & L2
After Treat	24	2	17	3,360		Profile	100%	
Upstream	103	4	59	27	41	Profile	100%	L4
Downstream	157	7	324	124	41	Profile	100%	L3
Crystal Mine								
Mine Waste	22,000	25,700	80,600	13,700	400	Definit	0.5%	CM
Lime Treatment	1,590	4	20	4	40	Profile	100%	

TABLE 2. Arrival Chemistries

Sample	Temp (°C)	pH (S.U.)	Alkal. (ppm)	Hard. (ppm)	Cond. (µS/cm)	D.O. (ppm)
CI	7.9	2.97	N/A	540	1792	9.1
CO	7.1	7.96	282	940	1737	8.5
R3	7.1	8.02	86	180	428	8.2
IBG	7.9	3.62	N/A	720	483	9.7
R2	9.9	7.97	200	350	648	8.2
R4	6.8	7.84	198	480	990	8.9
CM	11.0	3.21	N/A	N/A*	1322	9.7
PP	9.6	6.98	42	150	276	9.7
FIP	10.3	4.08	N/A	360	372	9.8
FFP	9.6	6.58	40	160	380	9.8
PT 3b	9.5	3.49	N/A	N/A*	678	9.9
LOB Down	10.2	7.12	20	20	62	9.7
Pt 3a	9.7	3.40	N/A	N/A*	752	9.9
LOB Up	9.3	7.43	20	12	53	9.9
MHRW	23.5	7.95	60	96	344	8.4

* Hardness could not be determined due to a color interference.
Alkalinity values marked with N/A could not be determined due to low pH. pH> 4.7 required.

<u>Samples and shortened designations.</u>

Calliope Columns Out (CO)
Calliope Columns In (CI)
Calliope Outflow Below Grade w/o Treatment (R3)
Calliope Influent into treatment, below grade (IBG)
Calliope Below Grade w/treatment (R2)
Calliope Above Grade pretreatment Outflow (R4)
Crystal Mine (CM)
Peerless Portal (PP)
Peerless Flow into Pile (FIP)
Peerless Flow from Pile (FFP
Lilly Orphan Boy Pt 3b (Pt 3b)
Lilly Orphan Boy Downstream (LOB Down)
Lilly Orphan Boy Pt 3a (Pt 3a)
Lilly Orphan Boy Upstream (LOB Up)

TABLE 3. Standard Operating Procedures for *Ceriodaphnia dubia* acute toxicity tests for

Superfund samples.

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	48 hr
Temperature	20°C ± 1°C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	30 ml (plastic cups)
Test Solution Volume	20 ml
Renewal of Test solution	Daily
Age of Test Organisms	Less than 24-hr-old
Number of Organisms/ per test chamber	5
Number of Replicate Chambers/Conc.	4
Number of Organisms/ Concentration	20
Feeding	none, fed while holding prior to test setup
Dilution Water	Moderately Hard Reconstituted Water
Endpoint	Mortality, LC50
Test Acceptability	≥ 90% survival in the controls

TABLE 4. Standard Operating Procedures for *Pimephales promelas* acute toxicity tests

for Superfund samples.

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	48 hr
Temperature	20°C ± 1°C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	175 ml (plastic cups)
Test Solution Volume	150 ml
Renewal of Test- solution	Daily
Age of Test Organisms	3 to 7 days ± 24 hr age range
Number of Organisms/ per test chamber	10
Number of Replicate- Chambers/Conc.	2
Number of Organisms/ Concentration	20
Feeding	Feed newly hatched brine shrimp prior to testing. Do not feed during the test.
Dilution Water	Moderately Hard Reconstituted Water
Endpoint	Mortality, LC50
Test Acceptability	≥90% survival in the controls

TABLE 5. Standard Operating Procedures for *D. magna* Survival and Growth Toxicity Tests for Superfund Samples.

TEST PARAMETER	CONDITION
Test Type	static-renewal
Test Duration	4 days
Temperature	25 ^{0}C (±1 ^{0}C)
Photoperiod	16 h light: 8 h dark
Test Chamber Size	60 ml
Test Solution Volume	50 ml
Renewal of Test Solution	daily
Age of Test Organisms	<24 hours old
No. Organisms/Test Chamber	5
No. Replicate Test Chambers	4
No. Organisms/concentration	20
Feeding Regime	0.3 ml algae and 0.2 ml alfalafa
Test Solution Aeration	None
Dilution Water	Moderately Hard Water
Endpoint	Survival and Mean Dry Weight
Test Acceptability	90% or greater control survival control growth 10X initial weight

TABLE 6. Results from toxicity tests with *Ceriodaphnia dubia.*

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Calliope	Control	20/20	0.23	0.18-0.30	0.05	19
IBG	0.0125	18/20				
	0.025	19/20				
	0.05	18/20				
	0.1	15/20				
	0.2	16/20				
	0.4	7/20				
	0.8	0/20				
Calliope	Control	19/20	0.07	0.06-0.07	0.05	14
Columns	0.0125	19/20				
In	0.025	20/20				
	0.05	16/20				
	0.1	1/20				
	0.2	0/20				
Calliope	Control	19/20	N/A	N/A		
Profiles	R2	20/20			>100%	
	R3	19/20			>100%	
	R4	19/20			>100%	
	CO	16/20			>100%	
Crystal	Control	20/20	0.04	0.03-0.05	0.015625[A]	21
Mine	0.0313	14/20				
	0.0625	1/20				
	0.125	0/20				
	0.25	0/20				
	0.5	0/20				

A) Estimated value, low concentration of 0.03125% was different from the control for survival.

TABLE 6. Results from toxicity tests with *Ceriodaphnia dubia*, cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Peerless	Control	20/20	<6.25	N/A	<6.25	N/A
FIP	6.25	0/20				
	12.5	0/20				
	25	0/20				
	50	0/20				
	100	0/20				
Peerless	Control	20/20	1.6	1.3-1.8	0.625	4
FFP	0.625	19/20				
	1.25	16/20				
	2.5	0/20				
	5	0/20				
	10	0/20				
Peerless	Control	20/20	1.2	1.1-1.2	0.78	10
Portal	0.78	19/20				
	1.57	1/20				
	3.125	0/20				
	6.25	0/20				
	12.5	0/20				
Lilly Orphan	Control	19/20	0.34	No Limits	0.25	8
Boy Pt 3a	0.25	17/20				
	0.5	0/20				
	1	0/20				
	2	0/20				
	4	0/20				

TABLE 6. Results from toxicity tests with *Ceriodaphnia dubia,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Lilly Orphan	Control	20/20	0.24	0.21-0.28	0.125	11
Boy Pt 3b	0.0313	20/20				
	0.0625	17/20				
	0.125	19/20				
	0.25	9/20				
	0.5	0/20				
Lilly Orphan	Control	19/20	33.9	30.1-38.3	25	18
Boy	6.25	18/20				
Downstream	12.5	18/20				
	25	17/20				
	50	1/20				
	100	0/20				
Lilly Orphan	Control	19/20	N/A	N/A	>100%	p=0.171
Boy	100%	16/20				
Upstream						

TABLE 7. Results from toxicity tests with *Pimephales promelas*.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Calliope	Control	20/20	9.2	6.8-12.4	1.25	21
IBG	1.25	20/20				
	2.5	13/20				
	5	15/20				
	10	10/20				
	20	0/20				
Calliope	Control	20/20	0.99	0.72-1.35	0.625	20
Columns	0.3125	17/20				
In	0.625	12/20				
	1.25	11/20				
	2.5	1/20				
	5	0/20				
Calliope	Control	20/20	N/A	N/A		
Profiles	R2	20/20			>100%	
	R3	20/20			>100%	
	R4	19/20			>100%	
Crystal	Control	20/20	0.37	0.24-0.46	0.25	24
Mine	0.0313	20/20				
	0.0625	20/20				
	0.125	19/20				
	0.25	15/20				
	0.5	6/20				

TABLE 7. Results from toxicity tests with *Pimephales promelas,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Peerless	Control	20/20	N/A*	N/A*	5	19
FFP	0.625	20/20				
	1.25	20/20				
	2.5	20/20				
	5	17/20				
	10	12/20				
restart 7-12	10	16/20	28.3	22.0-36.4	10	
	20	14/20				
	40	4/20				
Peerless	Control	20/20	39.2	31.9-48.3	12.5	14
Portal	6.25	20/20				
	12.5	19/20				
	25	16/20				
	50	8/20				
	100	0/20				
Lilly	Control	20/20	3.0	2.5-3.7	1	9
Boy Pt 3a	0.25	20/20				
	0.5	20/20				
	1	18/20				
	2	16/20				
	4	6/20				
Peerless	Control	20/20	8.1	7.0-9.4	<6.25	40
FIP	6.25	14/20				
	12.5	2/20				
	25	0/20				
	50	0/20				
	100	0/20				

* No LC50 (or Limits) could be generated from the first test. LC50 results based on data from second test with this sample.

TABLE 7. Results from toxicity tests with *Pimephales promelas,* cont'd.

Sample	Conc. (%)	Survival	LC50 (%)	Limits	NOAEL (%)	MSD %
Lilly Orphan	Control	20/20	6.1	5.1-7.3	4	17
Boy Pt 3b	4	17/20				
	8	5/20				
	16	0/20				
Lilly Orphan	Control	20/20	70.7	No Limits	50	26
Boy	6.25	20/20				
Downstream	12.5	20/20				
	25	20/20				
	50	20/20				
	100	0/20				
Lilly Orphan	Control	20/20	N/A	N/A	100%	p=1
Boy	100	20/20				
Upstream						

TABLE 8. Results from toxicity tests with *Daphnia magna*.

Sample	Conc.(%)	Survival (%)	$\bar{X}$ Wt.	CV %	Sur NOEC	Grw NOEC	MSD %	IC25
Calliope	Control	100	124	8.9	1.25	2.5	10	1.578
IBG	0.3125	100	115	8.7				
	0.625	100	116	2.6				
	1.25	100	116	2.6				
	2.5	45	28	64.3				
	5	0	0	0				
Calliope	Control	95	179	7.8	0.3125	<0.3125	12	0.349
Columns	0.3125	95	150	12.0				
In	0.625	10	11	200				
	1.25	0	0	0				
	2.5	0	0	0				
	5	0	0	0				
Profiles	Control	100	130	4.9	N/A	N/A		N/A
	R2	100	188	5.1				
	R3	100	154	5.2				
	R4	100	204	5.6				
Crystal	Control	100	152	9.9	0.25	0.03125	17	0.071
Mine	0.0313	100	131	8.4				
	0.0625	95	118	18.6				
	0.125	100	89	19.1				
	0.25	90	39	15.4				
	0.5	0	0	0				

TABLE 8. Results from toxicity tests with *Daphnia magna*.

Sample	Conc.(%)	Survival (%)	$\bar{X}$ Wt.	CV%	Sur NOEC	Grw NOEC	MSD %	IC25
Peerless	Control	100	140	14.0	1.25	1.25	11	2.24
Flow from	0.625	100	130	8.5				
Pile	1.25	100	128	6.3				
	2.5	85	98	17.3				
	5	40	24	62.5				
	10	0	0	0				
Lilly	Control	100	126	2.4	1	<0.25	7	0.653
Orphan	.25	100	116	3.4				
Boy Pt 3a	.5	95	105	4.8				
	1	80	70	11.4				
	2	15	30	86.7				
	4	0	0	0				

TABLE 9. Initial routine chemistries for *C. dubia, D. magna* and *P. promelas* tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(μS)	Temp.	(°C)
sxs	(%)	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Cont.	0	8.11	7.97	8.5	8.7	348	347	19.5	20.1
CI	0.013	7.44	7.91	8.3	8.5	347	349	19.5	20.2
	0.025	7.38	7.90	8.3	8.6	347	378	19.5	20.1
	0.05	7.35	7.88	8.2	8.6	346	349	19.6	20.1
	0.1	7.36	7.86	8.2	8.6	343	349	19.7	20.1
	0.2	7.40	7.87	8.3	8.6	346	353	19.5	20.0
	0.313	8.00	7.96	8.6	8.7	346	347	20.5	21.1
	0.625	7.84	7.94	8.7	8.6	349	349	20.5	21.0
	1.25	7.84	7.83	8.6	8.6	353	353	20.4	21.1
	2.5	7.35	7.57	8.6	8.6	362	361	20.4	21.1
	5	7.13	6.64	8.6	8.6	381	408	20.3	21.0
IBG	0.013	7.27	7.89	8.3	8.5	348	352	19.6	20.1
	0.025	7.25	7.88	8.3	8.6	348	347	19.8	20.1
	0.05	7.23	7.87	8.3	8.6	346	347	19.6	20.2
	0.1	7.24	7.92	8.2	8.6	343	346	19.7	20.2
	0.2	7.34	7.94	8.3	8.6	344	348	19.7	20.2
	0.4	7.25	7.98	8.7	8.5	344	345	20.1	19.1
	1.25	8.04	7.98	8.6	8.6	342	343	20.2	20.9
	2.5	7.99	7.88	8.6	8.6	341	342	20.2	21.0
	5	7.76	7.87	8.6	8.5	347	295	20.2	21.1
	10	7.55	7.70	8.7	8.4	344	296	20.1	21.0
	20	7.24	7.28	8.7	8.6	340	298	20.0	21.0
CO	100	7.96	8.14	8.5	8.9	1737	1725	20.7	19.5
R2	100	7.97	7.76	8.0	8.8	648	643	20.9	19.4
R3	100	8.02	8.07	8.2	8.7	428	426	20.9	19.4

R4	100	7.84	7.93	8.9	8.7	990	979	20.9	19.4

TABLE 9. Initial routine chemistries for *C. dubia, D. magna* and *P. promelas* tests. (cont'd)

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
CM	0.0313	7.94	8.12	8.4	8.5	345	347	19.3	20.9
	0.0625	8.04	8.12	8.4	8.6	345	347	19.1	21.0
	0.125	8.04	8.07	8.4	8.6	345	347	19.1	20.8
	0.25	8.01	8.02	8.4	8.5	346	348	19.1	21.0
	0.5	7.84	7.90	8.4	8.5	349	349	19.1	21.1
PP	0.78	7.84	7.84	8.5	8.6	353	349	20.4	20.1
	1.56	7.81	7.85	8.5	8.6	346	343	20.3	20.0
	3.125	7.85	7.82	8.5	8.6	347	347	20.4	20.0
	6.25	7.85	7.69	8.6	8.6	355	348	20.5	20.0
	12.5	7.69	7.52	8.5	8.6	348	350	20.4	19.9
	25	7.51	7.44	8.8	8.6	333	352	19.7	20.1
	50	7.17	7.16	9.0	8.6	310	315	19.4	20.3
	100	6.58	N/A	9.2	N/A	276	N/A	19.6	20.2
FIP	6.25	7.81	7.75	8.7	8.5	348	344	19.0	20.1
	12.5	7.68	7.71	8.7	8.5	342	341	19.1	20.2
	25	7.44	7.56	8.7	8.7	339	335	19.1	20.1
	50	7.04	7.25	8.8	8.9	329	323	19.1	20.0
	100	3.84	4.04	9.1	9.8	374	373	19.0	20.1
FFP	0.625	8.05	8.01	8.6	8.6	345	347	19.3	19.4
	1.25	8.06	8.02	8.6	8.6	347	347	19.2	19.3
	2.5	8.04	8.00	8.6	8.6	346	347	19.3	19.2
	5	7.96	7.97	8.6	8.7	350	348	19.3	19.2

	10	7.79	7.85	8.6	8.8	348	348	19.2	19.2

TABLE 9. Initial routine chemistries for *C. dubia*, *D. magna* and *P. promelas* tests. (cont'd)

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
Pt 3b	0.0313	8.07	7.81	8.7	8.3	351	335	20.0	20.0
	0.0625	8.02	7.84	8.7	8.3	349	339	19.8	19.9
	0.125	8.02	7.83	8.7	8.3	349	338	19.8	19.7
	0.25	8.02	7.90	8.7	8.3	347	335	19.6	19.8
	0.5	7.98	7.57	8.7	8.3	346	337	19.8	20.0
	4	7.75	7.85	8.6	8.4	354	309	19.8	21.1
	8	7.53	7.50	8.6	8.5	356	310	19.8	21.2
	16	7.23	7.04	8.7	8.5	363	324	19.7	21.2
LOB	6.25	8.05	8.04	8.7	8.4	329	330	20.6	19.5
Down	12.5	8.01	8.04	8.7	8.4	312	313	20.2	18.5
	25	7.94	8.00	8.7	8.4	280	277	20.4	19.5
	50	7.71	7.89	9.1	8.6	211	203	19.8	19.3
	100	7.06	7.11	9.6	8.9	55	50	19.0	19.3
Pt 3a	0.25	8.02	8.01	8.4	8.5	347	348	19.1	20.1
	0.5	8.04	8.07	8.4	8.5	346	348	19.2	19.9
	1	8.03	7.97	8.4	8.5	346	348	19.2	20.0
	2	7.97	7.96	8.4	8.5	347	349	19.3	19.9
	4	7.84	7.81	8.4	8.5	349	351	19.3	20.0
LOB Up	100	6.56	7.03	9.3	10.2	51	43	20.3	19.2

TABLE 10. Final routine chemistries from *C. dubia* tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
CI	cnt	8.13	8.09	8.7	8.5	356	351	20.7	20.8
	0.013	8.13	8.09	8.7	8.6	356	352	20.8	20.9
	0.025	8.14	8.11	8.7	8.6	354	352	20.9	20.7
	0.05	8.13	8.11	8.7	8.7	353	351	21.0	20.8
	0.1	8.12	8.11	8.7	8.6	350	351	20.9	20.6
	0.2	8.13	8.11	8.8	8.6	349	354	20.8	20.6
IBG	cnt	8.07	8.08	8.7	8.7	341	353	20.6	20.5
	0.013	8.03	8.08	8.7	8.7	355	353	20.7	20.6
	0.025	8.03	8.09	8.7	8.8	351	350	20.8	20.9
	0.05	8.02	8.09	8.7	8.7	348	349	20.8	20.8
	.1	8.04	8.09	8.7	8.7	345	348	20.8	20.7
	.2	8.04	8.09	8.7	8.8	346	347	20.8	20.7
	.4	8.10	8.12	8.7	8.7	347	352	20.2	21.0
	.8	8.11	8.15	8.5	8.6	347	353	20.2	21.0
Profile	cnt	7.97	8.02	8.6	8.7	345	351	20.4	21.0
	CO	8.45	8.41	8.6	8.5	1659	1648	20.5	21.0
	R2	8.17	8.18	5.0	7.9	626	643	20.5	20.9
	R3	8.15	8.07	8.7	8.5	454	424	20.5	20.8
	R4	8.11	8.24	7.3	8.2	957	957	20.5	21.0
CM	cnt	8.09	8.17	8.5	9.0	354	348	20.8	21.0
	0.0313	8.09	8.16	8.6	8.9	356	348	20.6	21.0
	0.0625	8.10	8.17	8.6	8.9	356	348	20.6	20.9
	0.125	8.10	8.16	8.6	8.9	358	349	20.6	20.8

	0.25	8.10	8.15	8.6	8.9	359	349	20.6	20.9
	0.5	8.00	8.12	8.6	8.9	359	350	20.6	21.0

TABLE 10. Final routine chemistries from *C. dubia* tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
PP	cnt	8.10	8.09	8.6	8.6	346	351	20.5	20.6
	0.78	8.02	8.08	8.6	8.6	348	350	20.7	20.5
	1.57	8.03	8.09	8.6	8.6	346	350	20.8	20.8
	3.125	7.99	8.07	8.6	8.6	347	350	20.8	20.7
	6.25	8.02	8.03	8.6	8.6	347	346	20.8	20.7
	12.5	7.96	8.00	8.6	8.6	346	353	20.7	20.7
FIP	cnt	8.12	N/A	8.6	N/A	352	N/A	20.5	N/A
	6.25	8.09	N/A	8.7	N/A	354	N/A	20.5	N/A
	12.5	8.02	N/A	8.7	N/A	353	N/A	20.6	N/A
	25	7.81	N/A	8.7	N/A	347	N/A	20.6	N/A
	50	7.60	N/A	8.7	N/A	337	N/A	20.6	N/A
	100	4.16	N/A	8.7	N/A	353	N/A	20.6	N/A
FFP	cnt	8.15	8.11	8.5	8.7	358	341	20.7	21.0
	0.625	8.13	8.12	8.6	8.7	356	350	20.7	21.0
	1.25	8.13	8.10	8.6	8.7	360	351	20.6	21.0
	2.5	8.12	8.09	8.7	8.7	358	351	20.7	20.9
	5	8.11	8.07	8.7	8.7	359	351	20.7	21.0
	10	8.08	8.02	8.6	8.7	358	352	20.9	20.8
Pt 3b	cnt	8.15	8.14	8.4	8.4	348	350	20.8	20.3
	0.0313	8.10	8.09	8.5	8.5	358	357	20.8	20.2
	0.0625	8.12	8.11	8.5	8.4	357	358	21.0	20.2

	.125	8.09	8.08	8.5	8.4	356	356	21.0	20.1
	.25	8.11	8.09	8.4	8.4	354	353	21.0	20.2
	.5	8.10	8.10	8.5	8.4	359	358	20.9	20.1

TABLE 10. Final routine chemistries from *C. dubia* tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
LOB	cnt	8.27	8.19	8.6	8.9	359	351	20.6	20.8
Down	6.25	8.24	8.16	8.6	8.8	338	333	20.8	20.7
	12.5	8.21	8.16	8.6	8.9	323	315	20.6	20.7
	25	8.10	8.11	8.6	8.9	290	280	20.6	20.7
	50	8.02	8.03	8.6	8.9	227	207	20.8	20.7
	100	7.80	7.59	8.6	8.9	72	60	20.7	20.7
Pt 3a	cnt	8.16	8.15	8.7	8.9	353	352	20.2	21.0
	0.25	8.13	8.16	8.7	9.0	356	353	20.1	20.9
	.5	8.12	8.17	8.8	8.9	360	355	20.3	20.9
	1	8.10	8.17	8.8	9.0	356	353	20.2	20.8
	2	8.07	8.14	8.8	8.9	358	353	20.2	20.8
	4	8.03	8.12	8.8	9.0	360	354	20.2	20.8
LOB	cnt	8.09	8.24	8.8	8.8	359	352	20.0	20.8
Up	100%	7.43	7.57	8.8	8.9	61	52	20.4	20.8

TABLE 11. Final routine chemistries from *P. promelas* toxicity tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
IBG	cnt	7.98	7.96	8.7	8.5	352	355	20.3	20.7
	1.25	8.06	8.10	8.7	8.5	354	357	20.3	20.4
	2.5	8.06	8.09	8.8	8.5	355	348	20.3	20.5
	5	8.00	7.97	8.5	8.7	350	354	20.5	20.5
	10	7.94	7.89	8.5	8.8	352	355	20.6	20.5
	20	7.76	7.65	8.5	8.8	344	350	20.6	20.5
CI	cnt	7.89	8.00	8.5	8.6	350	350	20.7	19.9
	0.313	7.94	8.05	8.5	8.7	356	372	20.6	20.2
	0.625	7.96	8.05	8.6	8.8	357	357	20.6	20.5
	1.25	7.94	8.00	8.6	8.8	360	362	20.8	20.5
	2.5	7.81	8.00	8.6	8.7	370	394	20.7	20.5
	5	7.60	N/A	8.6	N/A	387	N/A	20.8	N/A
Profile	cnt	7.96	8.04	8.7	8.6	347	352	20.5	20.5
	R2	7.84	7.97	8.4	8.4	424	430	20.5	20.5
	R3	8.01	8.16	4.4	6.9	625	642	20.4	20.5
	R4	8.00	8.23	6.4	7.6	950	978	20.4	20.5
CM	cnt	7.88	8.09	8.7	8.8	354	357	20.4	20.4
	0.0313	7.97	8.06	8.5	8.6	352	350	20.6	20.6
	0.0625	7.99	8.07	8.6	8.8	352	349	20.7	20.6
	0.125	7.97	8.08	8.6	8.8	353	351	20.5	20.7

	0.25	7.96	8.11	8.7	8.8	355	351	20.4	20.6
	0.5	7.95	8.09	8.7	8.8	356	351	20.4	20.6

TABLE 11. Final routine chemistries from *P. promelas* toxicity tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
PP	cnt	8.08	8.07	8.5	8.6	356	346	20.5	20.6
	6.25	7.96	8.05	8.5	8.7	356	360	20.5	20.5
	12.5	7.89	7.97	8.5	8.7	349	348	20.5	20.6
	25	7.86	7.92	8.6	8.7	341	341	20.5	20.6
	50	7.74	7.79	8.6	8.7	317	323	20.5	20.6
	100	7.48	N/A	8.5	N/A	355	N/A	20.3	N/A
FIP	cnt	7.84	8.07	8.6	8.7	358	353	20.3	20.4
	6.25	7.86	8.05	8.7	8.9	358	352	20.3	20.4
	12.5	7.82	8.01	8.7	9.0	354	345	20.3	20.4
	25	7.67	N/A	8.8	N/A	359	N/A	20.2	N/A
	50	7.49	N/A	8.9	N/A	356	N/A	20.2	N/A
	100	4.09	N/A	8.9	N/A	371	N/A	20.2	N/A
FFP	cnt	8.02	8.04	8.5	8.7	388	352	20.2	20.6
	0.625	7.98	8.08	8.5	8.7	354	353	20.3	20.5
	1.25	7.96	8.04	8.6	8.7	355	350	20.3	20.5
	2.5	7.93	8.04	8.6	8.7	353	351	20.3	20.5
	5	7.94	8.01	8.6	8.7	359	354	20.3	20.5
	10	7.85	8.00	8.6	8.7	357	353	20.3	20.6
	10	7.87	7.87	8.3	8.6	356	349	20.5	20.6
	20	7.82	7.80	8.3	8.6	357	353	20.6	20.5
	40	7.69	7.70	8.3	8.6	357	359	20.6	20.4

Pt 3b	cnt	7.88	7.81	8.2	8.5	355	338	20.6	20.5
	4	7.88	7.84	8.3	8.7	358	351	20.4	20.5
	8	7.85	7.79	8.4	8.7	360	359	20.6	20.5
	16	7.60	7.56	8.5	8.7	367	365	20.5	20.5

TABLE 11. Final routine chemistries from *P. promelas* toxicity tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
LOB	cnt	8.01	8.10	8.7	8.6	369	370	19.5	20.7
Down	6.25	8.04	8.08	8.7	8.7	340	344	20.4	20.6
	12.5	8.01	8.06	8.8	8.7	318	327	20.3	20.6
	25	7.97	8.01	8.7	8.6	289	318	20.2	20.6
	50	7.92	7.99	8.6	8.7	217	266	20.4	20.7
	100	7.61	N/A	8.7	N/A	60	N/A	20.5	N/A
Pt 3a	cnt	7.97	7.97	8.5	8.6	355	358	20.2	20.6
	0.25	7.96	8.03	8.5	8.8	353	350	20.4	20.5
	0.5	7.97	8.02	8.6	8.7	358	351	20.4	20.5
	1	7.94	8.03	8.5	8.7	357	351	20.3	20.5
	2	7.92	8.00	8.6	8.7	360	356	20.3	20.6
	4	7.88	7.98	8.6	8.7	359	355	20.4	20.7
LOB	cnt	8.13	8.11	8.6	8.7	351	348	20.4	20.6
Up	100	7.65	7.77	8.7	8.7	52	46	20.3	20.6

TABLE 12. Final routine chemistries from *D. magna* toxicity tests.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	low	high	low	high	low	high	low	high
CI	cnt	7.90	8.15	7.8	8.4	356	361	24.0	24.6
	0.313	7.85	7.97	7.8	8.0	351	362	24.2	24,6
	0.625	7.90	8.14	8.0	8.4	357	361	24.2	24.9
	1.25	7.91	7.95	8.0	8.0	366	366	24.1	24.8
	2.5	N/A	7.88	N/A	8.0	N/A	375	N/A	24.2
	5	N/A	7.42	N/A	8.2	N/A	421	N/A	24.3
IBG	cnt	7.65	8.05	7.7	8.1	357	369	24.2	24.5
	0.313	7.67	8.11	7.7	8.1	353	359	24.3	24.6
	0.625	7.64	8.07	7.6	8.1	353	359	24.4	24.5
	1.25	7.69	8.01	7.6	8.1	354	357	24.1	24.5
	2.5	7.80	8.04	7.9	8.1	353	356	24.3	24.5
	5	7.82	7.97	8.0	8.1	351	368	24.1	24.5
Profile	cnt	7.77	8.02	7.6	8.2	357	366	24.1	24.7
	R2	8.07	8.23	7.0	7.6	428	443	24.2	24.6
	R3	7.74	7.98	7.5	7.9	629	639	24.1	24.6
	R4	8.14	8.27	7.3	7.8	946	969	24.2	24.6
CM	cnt	7.83	8.31	7.9	8.3	350	360	24.4	24.8
	.0313	7.85	8.22	7.8	8.3	353	357	24.4	24.6
	.0625	7.85	8.10	7.8	8.2	351	359	24.2	24.6

	.125	7.88	8.06	8.0	8.0	355	363	24.1	24.5
	.25	7.91	8.02	8.0	8.0	353	357	24.0	24.6
	.5	7.94	8.00	8.0	8.0	356	359	24.6	24.6

TABLE 12. Final routine chemistries from *D. magna* toxicity tests, cont'd.

	Conc.	pH	(SU)	D.O.	(ppm)	Cond.	(µS)	Temp.	(°C)
sxs	(%)	low	high	low	high	low	high	low	high
FFP	cnt	7.87	8.02	7.8	8.3	353	368	24.2	24.6
	0.625	7.86	8.00	7.7	8.3	352	362	24.3	24.7
	1.25	7.82	7.97	7.7	8.2	351	366	24.3	24.8
	2.5	7.82	7.96	7.7	8.2	352	361	24.2	24.8
	5	7.83	8.03	8.0	8.2	354	364	24.5	24.9
	10	7.82	8.00	8.1	8.1	354	372	24.3	24.4
PT 3a	cnt	7.86	8.13	7.7	8.5	353	366	24.4	24.6
	0.25	7.84	8.12	7.8	8.5	354	358	24.3	24.6
	0.5	7.88	8.10	7.9	8.5	350	360	24.3	24.5
	1	7.81	8.08	7.7	8.4	351	358	24.2	24.5
	2	7.84	8.05	7.6	8.4	352	365	24.2	24.5
	4	7.92	7.96	8.2	8.5	352	364	24.2	24.5

TABLE 13. Chemical Analyses of Samples for Selected Metals
Concentration represents the sample at the time toxicity tests were run, not at time of collection.

Concentration in µg/l:Filtered Samples

	Al	Cd	Cu	Zn	Mn	Pb
Lilly Orphan Boy						
L1 PT3a	7,210	189	310	15,200	5,580	130
L2 downstream	121	17	4	280	201	<4
L4 upstream	84	<1	2	16	611	<4
L3 PT3b	6,820	180	300	14,700	5,620	121
Peerless						
PP Portal	294	12	195	1,680	4,390	23
P1 Flow into Pile	1,390	36	760	5,090	9,070	48
P2 Flow out of Pile	757	35	121	5,470	10,100	161
Calliope						
C1 Reactor 3 outflow	69	14	26	1,230	1,640	<4
C2 Influent to reactors	6,620	26	1,570	6,430	2,560	9
C3 Reactor 2 outflow	44	2	28	590	1,130	<4
C4 Reactor 4 outflow	24	<0.001	6	1,370	1,630	<4

CI Column In	41,000	113	10,900	26,600	7,810	<4
CO Column Out	75	<0.001	5	<1	10	15
CM Crystal Mine	19,000	1,280	19,700	53,000	15,300	405

TOXICITY TEST REPORT
Project: North Fork Clear Creek Toxicity Tests, September, 1998.

Date: August 13, 1998 (Revised 1/22/99)
Mark Smith, SBI Work Assignment Leader & Jim Lazorchak, EPA Work Assignment Manager

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INTRODUCTION

The North Fork of Clear Creek (NFCC) is a site located in the Central Region of Colorado, west of Denver. It is in the Rocky Mountain region and serves as one of the main tributaries to Clear Creek, part of the South Platte River drainage basin. Concerns over the site are not completely known, but the sources of impacts are mines, both abandoned and operating, with the resulting heavy metals and low pH impacts. The purpose behind this study is to establish a baseline for the extent of contamination (toxicity) for both the water column and the sediment at selected sites throughout the stream reach and tributaries. This study should also aid in tracking the trends (changes) in the watershed during the study. Seven of the water column samples were tested using the *Daphnia magna* four day survival and growth toxicity test method. The remaining two samples were tested using a *C. dubia* 48 hour acute toxicity test method. The sediment samples were analyzed with a 10-day sediment toxicity test using *Hyalella azteca,* as well as a 4-day sediment toxicity test using *D. magna*. The *D. magna* method is experimental, as is the *P. promelas* 8-day sediment embryo-larval test that was also used. Background data does exist for this site, due to testing of various segments of the stream for the Superfund Program.

METHODS and MATERIALS

Water column and sediment toxicity samples were collected at NFCC by USEPA field personnel and shipped to SBI at the Newtown laboratory. These samples were collected over 9-20-97 and 9-22-97 and were received at the laboratory 9-23-97. Arrival chemistries were determined for all samples on receipt, Table 1.

The tests with the water column samples were started 9-23-97. Two water column samples, SW-05 and SW-07A, were analyzed using an acute *C. dubia* test procedure. These samples were collected as part of a separate study into the remediation of the Argo Tunnel, Idaho Springs, CO. The acute test method used a 48 hour test duration, with daily test solution renewal, Table 10. Test solution volume was 20 mls and the test used <24 hour old *C. dubia* at the start.

The remaining 7 samples were analyzed using the *D. magna* 4-day survival and growth test method. The *D. magna* tests, Table 7, used a four day test duration, with a daily test solution renewal. They were fed daily 0.3 ml of *Selenastrum capricornutum* algae (80-100 million cells/ml) and 0.2 ml blended alfalfa extract (7.5 g alfalfa/1000 ml deionized water). The test solution volume was 50 ml. The tests were started using <24 hour old *D. magna*, in a 12-hour age range. A complete listing of this procedure is contained in Table 7.

The sediment toxicity testing procedures used the standard 10-day *H. azteca* survival and growth sediment test method, Table 8. This was a static-renewed test and used 7 day old *H. azteca*. The water was renewed and the test animals were fed daily with 2 ml of a mixture of a 1:1 suspension of *S. capricornutum* (80-100 million cells/ml) and alfalfa extract (5 g alfalfa/500 ml deionized water). In addition to the *H. azteca*, *D. magna* were also exposed in this procedure, Table 7. This was done by adding five *D. magna* to each test beaker, so that the two species were exposed concurrently. The *D. magna* underwent the same procedures as the *H. azteca*, for four days. At the end of the four day duration, the *D. magna* were pipetted from the test containers, for final survival and dry weights.

The final sediment toxicity testing procedure was a 7-day *P. promelas* embryo-larval test method, Table 9, currently under development. This method involved exposing the fish embryo directly on the surface of the sediment, allowing for interaction between the test animal and the sediment. This method uses 40 ml of sediment and 60 ml of overlying water. The water is replaced daily and the live/dead/hatched embryo and larvae are counted.

The water column tests used moderately hard reconstituted water (MHRW), with selenium added, as the standard control and dilution water. The sediment toxicity tests used reformulated moderately hard reconstituted water (RMHRW) as the overlying water. Both were prepared using a Millipore Super-Q® deionized water system. The control sediment was a top soil control (TS Control) prepared by grinding commercial top soil in a blender and then sieving the material through a # 8 sieve (2.36 mm) to remove the debris. This material was stored dry, and wetted using RMHRW (100 ml sediment/45 ml water).

The data were analyzed using several methods. The LC50 values were determined using Trimmed Spearman-Karber, v1.5. The NOEC and LOEC levels, as well as all t-tests, were determined using Sigma Stat®. All ANOVA and t-test alpha levels were 0.05. All growth determinations are based on mean dry weight.

<u>RESULTS and DISCUSSION</u>

Results from both the water column and sediment toxicity tests indicated both acute and chronic toxicity at the test sites. The results from SW49B indicates it is acceptable as an upstream reference site, for both water column and sediment toxicity testing (with *H. azteca*).

Daphnia magna Water Column Toxicity Tests

Tests with the water column sample using the *D. magna* 4-day test method started 9-23 ran into a problem with control survival. We do not know what caused this problem, but the controls for all tests died on day 2. This resulted in the need to restart all tests with new test animals. These tests, which included samples SW36, SW37, SW39, and SW43, were restarted 9-28-97. A modified definitive dilution series was used, 12.5% to 100%. The second set of tests did meet the control survival criteria, as well as the growth criteria. The initial weight of the animals used to start these tests was 8.7 µg, so the 10X the initial weight criteria was 87 µg. The remaining tests, with SW40, SW48 and SW49B, were started 9-24-97. SW40 and SW48 used a dilution series of 6.25% to 100%. SW49B was analyzed as a 100% only, profile sample. The *D. magna* used to start these tests weighed 7.5 µg, so the 10X the initial weight control growth criteria for these tests is 75 µg. All results are contained in Tables 2 and 3.

______For sample SW36, control survival was 100% and growth in the control was 106 µg. The survival NOEC value for this sample was 25%, with an LC50 value of 46.6%. The growth NOEC value was 12.5% and the IC25 value was 16.7%

For sample SW37, control survival was 95% and growth in the control was 90 µg. The survival NOEC value was 25% and the LC50 value was 47.3%. The growth NOEC value was 25% and the IC25 was 28.1%

For sample SW39, control survival was 95%, with a control growth of 98.25 µg. The survival NOEC value was 25%, with an LC50 value of 52.8%. The growth NOEC value was 12.5% and the IC25 value was 20.2%.

For sample SW40, control survival was 100%, with a control growth of 128 µg. The survival NOEC value was 25% and the LC50 value was 51.8%. The growth NOEC value was <6.25%, with an IC25 value of 15.8%.

For sample SW43, control survival was 100%, with a control growth of 92.5 µg. The survival NOEC value was 25%, with an LC50 value of 49.1%. The growth NOEC value was 12.5%, with an IC25 value of 16.1%.

For sample SW48, control survival was 100%, with a control growth of 90 µg. The survival NOEC value was 50%, with an LC50 value of 65.1%. The growth NOEC value was 12.5% and the IC25 value was 16.1%.

For the SW49B profile sample, the control survival was 100%, with a growth of 90 µg. Survival in the SW49B 100% sample was 100% and the growth of the *D. magna* in the sample was 146 µg.

In summary, the samples collected from SW36, SW37, SW39, SW40, SW43 and SW48 showed both acute and chronic toxicity in the *D. magna* 4-day survival and growth test. At a minimum, the 100% sample was acutely toxic, with the 50% sample being acutely toxic in five of these six samples. All samples also showed a chronic toxicity, with five of the six growth NOEC values being 12.5% or less. SW49B was not toxic, with the survival being equal to that of the control, while the growth of the *D. magna* in the sample exceeded the growth in the control by over 35%. SW49B is acceptable as an upstream reference site. The data indicates the stream is picking up toxicity somewhere above SW48, but below SW49B. As the sampling stations move downstream, the toxicity remains.

Hyalella azteca Sediment Toxicity Tests

The survival of the control animals (68.75%) in this test was below the minimum acceptability criteria of 80%. The results from the upstream reference site, SW49B will be used for all data analysis. Results from sediment toxicity testing with *H. azteca* indicate only one site is acutely toxic, SW36 (survival = 51.25%). Analyzing this data against that of the upstream sample, SW49B (survival = 98.75%) with a t-test ($\alpha = 0.05$) resulted in a pvalue of 0.0002, which indicates the survival in this sample is statistically different from that of the control. For growth, four of the samples (SW07A, SW36, SW39, and SW40) were determined to have growth statistically different from that of SW49B (Table 4).

Daphnia magna Sediment Toxicity Tests

None of the samples were determined to be toxic based on the results of the *D. magna* sediment toxicity tests. Survival in the control was 85%, with a growth of 177.7 µg. The lowest sample survivals were 55% (SW43) and 60% (SW37). Neither was determined to be statistically different from the control based on a t-test, $\alpha = 0.05$ (Table 5). The same was true for the growth endpoint, the growth in six of the nine samples exceeding the control growth. Analysis of the remaining three found none of the three were statistically different from the control growth. The lowest growth was in SW43, 139.3 µg (Table 5). The pvalue for this sample, when compared to the control, was 0.3633. NOTE: The survival of the *D. magna* in the control sediment is below the 90% control survival criteria used in the water column test. Since the use of the *D. magna* 4-day test procedure with sediments is new, insufficient data currently exists to determine a control survival criteria for the sediment test method. It is possible that for this method, a control survival criteria of 80% is appropriate. Since we are not certain at this point, it is best interpret these results with caution.

P. promelas Sediment Toxicity Tests

No toxicity was found in the sediment samples tested using the FHM embryo-larval sediment test method. Survival in the control sample was 60%, below the minimum survival criteria of 80%. The best survival was in SW36 (90%), so this was used as the comparison for estimating toxic sediments. Six of the nine sediments exceeded 75% survival, and were determined to not be impacted. These six were the lowest downstream samples on NFCC, SW36, SW37, SW39, SW40, and SW42. The two upstream samples, SW-48 and SW49B, had the lowest overall survival on

NFCC, 67.5% and 72.5% respectively. Lowest survival for any site tested was SW05, with a survival of 40%. The variability in the sample was excessive, so it was not determined to be statistically different from the control. The pvalue was 0.599, on the border of being significantly different.

In summary, the sediment toxicity data indicate the only sediment on NFCC acutely toxic to the *H. azteca* was SW36, the station farthest downstream. For growth, SW39 and SW40 were determined to be different from the upstream control, SW49B. The *D. magna* and *P. promelas* sediment tests did not detect toxicity in any of the samples.

Argo Tunnel Toxicity Testing

The toxicity of the two stations from mainstem of Clear Creek indicate the stream is not acutely toxic upstream at SW07A, *C. dubia* LC50 >100%. At the next downstream station, acute toxicity increases significantly, with the *C. dubia*, LC50 = 19.8%. Neither sediment sample was acutely toxic to the *H. azteca*, with survivals of 96.25% in SW05 and 80% in SW07A. Growth in SW05 was also acceptable, 68 µg, while the growth in SW07A was determined to be statistically different from the SW49B control, 52 µg versus 83 µg. No toxicity was detected using the *D. magna* sediment toxicity testing method. For the FHM embryo-larval sediment test, the survival in SW05 was 40%, but the pvalue was 0.0599, above the 0.05 significance level. However, examining the coefficient of variance for this test indicates excessive variability. So, while the data analysis indicates no difference from the control, a survival of 40% does indicate the potential for some contaminate, since the survival in the upstream sample, SW07A, was 75%. This sample (SW05) could possibly be toxic to the FHM embryo, but more data is required for a definitive answer. As it stands, the water column is acutely toxic to the *C. dubia*, while the sediments may be toxic to the FHM embryo.

Table 1. Arrival Chemistries

SAMPLE	DATE RECEIVED	TEMP (°C)	pH (S.U.)	ALK (PPM)	HARD (PPM)	COND (S/cm)	D.O. (PPM)
SW05	9/23/97	1.4	7.24	26	68	150	9.7
SW07A	9/23/97	1.3	7.25	30	60	132	9.9
SW36	9/23/97	1.1	6.93	12	148	347	9.4
SW37	9/23/97	1.4	6.68	8	139	317	10.4
SW39	9/23/97	1.2	6.40	9	151	345	10.2
SW40	9/23/97	1.3	6.24	8	153	334	9.1
SW43	9/23/97	1.1	6.20	10	132	300	9.9
SW48	9/23/97	1.1	7.14	24	48	109	9.6
SW49B	9/23/97	1.2	7.24	26	68	150	9.9
MHW	9/21/98	23.2	7.94	62	94	331	8.2

MHW Moderately Hard Reconstituted Water-Control for Water Column Tests

Table 2. *D. magna* Survival and Weight Data, *C. dubia* survival data Water Column Toxicity Tests.

SAMPLE ID	CONC. %	SURV.%	C.V. %	Wt. (ug)	C.V. %
SW05	control	95	10.5	N/A	N/A
C. dubia	3.125	100	0	N/A	N/A
acute	6.25	100	0	N/A	N/A
	12.5	95	10.5	N/A	N/A
	25	20	115.5	N/A	N/A
	50	10	200	N/A	N/A
SW07A	control	100	0	N/A	N/A
C. dubia	6.25	100	0	N/A	N/A
acute	12.5	100	0	N/A	N/A
	25	100	0	N/A	N/A
	50	100	0	N/A	N/A
	100	90	12.8	N/A	N/A
SW36	control	100	0	106	13.9
	12.5	100	0	90.5	3.3
	25	100	0	57.5	13.1
	50	40	91.3	19	96.3
	100	0	0	0	0
SW37	control	95	10.5	90	12.1
	12.5	95	10.5	79.5	3.2
	25	95	10.5	74.6	11.2
	50	40	70.7	17	75.9
	100	0	0	0	0
SW39	control	95	10.5	98.25	14.1
	12.5	95	10.5	93.5	9
	25	85	11.8	61.5	17.9
	50	65	29.5	27.5	39.6
	100	0	0	0	0

Table 2. *D. magna* Survival and Weight Data, Water Column Toxicity Tests

SAMPLE ID	CONC. %	SURV.%	C.V. %	Wt. (ug)	C.V. %
SW40	control	100	0	128	5.8
	6.25	100	0	99.5	17.6
	12.5	100	0	101	15
	25	100	0	84	24.3
	50	55	34.8	40	33.9
	100	0	0	0	0
SW43	control	100	0	92.5	11.9
	12.5	95	10.5	95.5	11.8
	25	85	11.8	65.5	25.2
	50	60	38.5	12.3	92.4
	100	0	0	0	0
SW48	control	100	0	90	12.4
	6.25	95	10.5	92.5	11.2
	12.5	100	0	80.5	9.4
	25	95	10.5	39	39.4
	50	90	22.2	34.5	26.5
	100	0	0	0	0
SW49B	control	100	0	90	12.4
	100	100	0	146	13.5

Table 3. *D. magna* and *C. dubia* NOEC, LOEC, LC50 and IC25 Values, Survival and Growth.

		Survival	(%)		Growth(%)	
Sample	NOEC	LOEC	LC50	NOEC	LOEC	IC 25
SW05	12.5	25	19.8	N/A	N/A	N/A
SW07A	>100	>100	>100	N/A	N/A	N/A
SW36	25	50	46.6	12.5	25	16.7
SW37	25	50	47.3	25	>25	28.1
SW39	25	50	52.8	12.5	25	20.2

SW40	25	50	51.8	<6.25	<6.25	15.8
SW43	25	50	49.1	12.5	25	22.8
SW48	50	100	65.1	12.5	50	16.1
SW49B*	>100	>100	>100	>100	>100	>100

Table 4. Survival and Growth Data for *H. azteca* Sediment Toxicity Tests.

Sample	Survival %	C.V.%	p value	Wt. ug	C.V.%	p value
TS CONT	68.75	15	N/A	67	19.2	N/A
SW05	96.25	5	0.4476	68	18	0.2178
SW07A	80	24.5	0.0878	52	31.7	0.0483
SW36	51.25	25.7	0.0002	58	9.4	0.0381
SW37	95	4.3	0.1733	66	29.9	0.2491
SW39	91.25	15.7	0.3654	51	7.5	0.0151
SW40	78.75	19	0.0551	46	22.5	0.0125
SW43	95	4.3	0.1733	61	6.2	0.0583
SW48	93.75	8	0.3401	61	12.3	0.0671
SW49B*	98.75	2.5	N/A	83	22.2	N/A

*Upstream reference site used for data analysis with t-tests.

Table 5. Survival and Growth data for *D. magna* Sediment Toxicity Test.

Sample	Survival %	C.V.%	p value	Wt. µg	C.V.%	p value
TS CONT	85	22.4	N/A	177.7	24.6	N/A
SW05	85	11.8	0.7797	215.8	14.2	0.2045
SW07A	80	28.8	0.8398	211	14.6	0.2591
SW36	100	0	0.1457	171.5	8.5	0.7954
SW37	60	38.3	0.1153	211.5	20.5	0.3148
SW39	100	0	0.1457	196.5	9.1	0.4579
SW40	85	22.4	1	162.8	18.4	0.5919
SW43	55	63.6	0.2419	139.3	46.6	0.3633
SW48	90	22.2	0.6496	197.8	21.4	0.5359
SW49B	80	20	0.6237	188	6.7	0.6684

Table 6. Survival Data for *P. promelas* Sediment Toxicity Test.

Sample	Survival %	C.V.%	p value*
MTSCont.	60	13.6	N/A
SW05	40	102.1	0.0599
SW07A	75	7.7	N/A
SW36	90	15.7	N/A
SW37	75	23.1	N/A
SW39	87.5	14.4	N/A
SW40	80	10.2	N/A
SW43	87.5	10.9	N/A
SW48	67.5	22.2	0.0718
SW49B	72.5	13.2	N/A

* Comparisons made using SW36 (sample with best survival) in a t-test.

TABLE 7. Standard Operating Procedures for *Daphnia magna* Survival and Growth Toxicity Tests for Superfund Samples.

TEST PARAMETER	CONDITION
Test Type	static-renewal
Test Duration	4 days
Temperature	25 ^{0}C ($\pm$1 ^{0}C)
Photoperiod	16 h light: 8 h dark
Test Chamber Size	60 ml
Test Solution Volume	50 ml
Renewal of Test Solution	daily
Age of Test Organisms	< 24 hrs old
No. Organisms/Test Chamber	5
No. Replicate Test Chambers	4
No. Organisms/concentration	20
Feeding Regime	0.3 ml algae & 0.2 ml alfalfa
Test Solution Aeration	None
Dilution Water	Moderately Hard Water + Selenium
Endpoint	Survival and Mean Dry Weight

Test Acceptability	90% or greater control survival control growth 10X initial weight

TABLE 8. Standard Operating Procedures for *Hyalella azteca* acute toxicity tests for Superfund samples. (Also for multi species test with *Daphnia magna*)

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	10 days
Temperature	23 ^{0}C ± 1 ^{0}C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	200 ml
Sediment Volume	75 ml
Overlying Water Volume	125 ml
Renewal of Test solution	daily
Age of Test Organisms	7-days old, 24 hour age range
Number of Organisms/ per test chamber	20 (5 <24 hr old *D. magna*)
Number of Replicate Chambers/Conc.	4 (Same for D. magna)
Number of Organisms/ Concentration	80 (20 *D. magna*)
Feeding	2 ml algae/alfalfa
Dilution Water	Reformulated Moderately Hard Reconstituted Water
Control Sediment	Potting Soil
Endpoint	Mortality, difference from control Growth, difference from control
Test Acceptability	> 80% survival in the controls *D. magna*, > 90% and final weight 10X initial

TABLE 9. Standard Operating Procedures for *Pimephales promelas* embryo-larval sediment toxicity test.

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	7 days
Temperature	25 ^{0}C ± 1 ^{0}C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	100 ml
Sediment Volume	25 ml
Overlying Water Volume	75 ml
Renewal of Test solution	daily
Age of Test Organisms	24-48 hour old embryo
Number of Organisms/ per test chamber	10
Number of Replicate Chambers/Conc.	4
Number of Organisms/ Concentration	40
Feeding	none
Dilution Water	Reformulated Moderately Hard Reconstituted Water
Control Sediment	modified top soil
Endpoints	Mortality, difference from control Hatch ability and abnormal embryo (terata)
Test Acceptability	> 80% survival in the controls

TABLE 10. Standard Operating Procedures for *Ceriodaphnia dubia* acute toxicity tests for Superfund Samples

TEST CRITERIA	SPECIFICATIONS
Test Type	Static-renewal
Test Duration	2 days
Temperature	20 ^{0}C ± 1 ^{0}C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	30 ml
Test Solution Volume	15 ml
Renewal of Test solution	daily
Age of Test Organisms	<24 hours old
Number of Organisms/ per test chamber	5
Number of Replicate Chambers/Conc.	4
Number of Organisms/ Concentration	20
Feeding	Fed while holding, prior to start of test.
Dilution Water	Moderately Hard Reconstituted Water
Endpoints	Survival, LC50
Test Acceptability	> 90% survival in the controls